MICROBIC VIRULENCE AND HOST SUSCEPTIBILITY IN PARATYPHOID-ENTERITIDIS INFECTION OF WHITE MICE.

IX. The Relationship of Dosage to Mortality Rate, Survival Time, and Cage Population.

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Early in the course of our studies on the epidemiology of mouse typhoid, Flexner[1] and Amoss,[2] discussing epidemics in general and experimental mouse typhoid epidemics in particular, drew attention to a possible relationship existing between dosage and the phenomenon of recurrent epidemic waves in an infected population. From the deaths occurring in his so called mouse village, where the spread of infection from cage to cage through the attendant who fed the mice and cleaned the cages was left to accident, Amoss was able to construct a typical epidemic curve, to which subsequent epidemic curves, following the addition of new mice to the infected population, closely conformed. Webster,[3] working with the same epidemic strain of mouse typhoid bacilli, demonstrated later that when virulence and population susceptibility were kept constant, dosage exerted a direct and quantitative effect upon mortality. By administering to each mouse a dose of 5,000,000 bacteria he was able to obtain, at will, mortality curves which could be superimposed upon Amoss' experimental epidemic curves. The same result was obtained indirectly by varying the number of infected mice in contact with normal unexposed animals. From these observations he concluded that "with a given susceptible mouse population and a certain strain of mouse typhoid bacilli

[1] Flexner, S., *J. Exp. Med.*, 1922, xxxvi, 9.
[2] Amoss, H. L., *J. Exp. Med.*, 1922, xxxvi, 45.
[3] Webster, L. T., *J. Exp. Med.*, 1923, xxxvii, 269.

[in this case M. T. II] the sporadic and epidemic prevalences of mouse typhoid are determined by the spacial and quantitative distribution of the bacilli." He suggested, moreover, that the duration of life of normal contact mice added to an infected population may depend not only on their individual susceptibility, but on the degree of distribution of the bacilli in their environment.

In the experiments to be described, we have attempted to test these conclusions by determining in a mouse population infected with mouse typhoid the relationship between the fluctuating epidemic waves of mortality, and the available dosage, or number of mouse typhoid bacilli in the cage.

Method.

As a nucleus for these epidemics, there was arbitrarily chosen a population of ten mice of our Rockefeller Institute strain, each being injected *per os* with the usual standard dose of M. T. II (about 5,000,000 bacilli). This population was increased at a constant rate by the daily addition of two normal contact mice, also of our Institute strain. Two separate epidemics were thus studied at the same time, the mice of each epidemic being kept in a wire-topped zinc box measuring $5 \times 7 \times 10$ inches. In each of the two boxes, as bedding for the mice, was placed a weighed quantity of very fine sawdust, which served also as a medium for the accumulation of bacteria excreted by the infected mice in the feces and urine. Preliminary experiments had shown that mouse typhoid bacilli, added in pure culture to such sawdust, were able to survive for periods of at least 3 or 4 days.

Besides controlling the population, we arbitrarily affected the dosage by periodically transferring the mice, in Box 1 every 2nd day and in Box 2 every 3rd day, to clean boxes containing fresh sawdust. Theoretically this might be expected to lead, in Box 1, to rapid and small fluctuations in the number of bacilli present, which would rise to a height every 2 days, and in Box 2 to less rapid and considerably greater fluctuations, reaching a peak every 3 days.

The following procedure was adopted for determining the number of mouse typhoid bacilli excreted by the mice and present in the sawdust. The whole volume of sawdust (50 gm.) was removed from the box and placed in a sterile bottle containing 500 cc. of beef infusion broth, after which the dirty boxes were subjected to steam sterilization. The bottle containing the sawdust-broth mixture was tightly corked and shaken in a shaking machine for 1 hour, in order to mix the material thoroughly with the broth and enable the masses of fecal material to soften. After shaking part of the supernatant broth was placed in large centrifuge tubes and whirled at low speed for a very short period—perhaps 30 seconds with the motor running—to throw down the heaviest particles which sometimes made it impossible to draw the uncentrifuged broth into the pipette. Dilution

plates were then poured in green dye agar, the dilutions of broth used for plating being 1:20, 1:200, 1:2000, and 1:20,000. These plates were incubated overnight at 37°C. and were counted the next morning.

For counting the plates a special technique was devised. First the total number of organisms present on the plate was counted in the usual manner. Frequently these all appeared to be typical for the mouse typhoid bacillus, but sometimes other organisms also developed, chiefly a colony resembling *Bacillus aerogenes*. When this happened it was necessary to estimate the number of mouse typhoid bacilli present. This was done by counting all the surface colonies, among which the colonies of M. T. II could be easily distinguished. The ratio of the mouse typhoid surface count to the total surface count was assumed to be the same as the ratio of the total typhoid count to the total count. From this ratio the total mouse typhoid count could be easily estimated. Having ascertained the number of mouse typhoid bacilli present in each cc. of the broth, it was necessary merely to multiply the result by 500 to obtain roughly the total number present, on this basis, in the whole 500 cc. of broth, and hence in the 50 gm. of sawdust taken from each box. The log of the value thus obtained was used in plotting a curve of the varying numbers of mouse typhoid bacilli present in each box and potentially available to the mice during the whole of the experimental period.

With the exception of the intervals at which the dosage was arbitrarily modified by changing the sawdust in the box, the mice in both epidemics received precisely the same treatment. They were fed daily on bread soaked in pasteurized Grade B milk, and two normal mice of our Rockefeller Institute strain were added daily as contacts. Each of these contact mice was marked with picric acid so that it could at any time be identified. At 9 o'clock each morning the boxes were gone over and all dead mice were recorded. Whenever possible, all these mice were autopsied and their organs cultured, but in about 73 per cent of the deaths occurring in each box this was rendered impossible through decomposition of the dead mice, or through the tendency of the living ones to eat their dead companions. As the experiment progressed, we hit upon the expedient of autopsying the mice as soon as they were found dead, and then returning them to the box in order that the conditions of the experiment might not be changed by removing this important source of infection from mouse to mouse.

During the second half of the experimental period, from about the middle of March on, difficulties were encountered in estimating the number of mouse typhoid bacilli present in the boxes, and it was eventually discovered that the original epidemic strain (M.T. II) had been almost entirely replaced by M.T. I (*Bacillus enteritidis*). This organism, antigenically dissimilar from our M.T. II strain, is known to be, to a mild degree, endemic among the breeding room mice of our Institute stock, and probably gained entrance to the boxes through the inadvertent inclusion of fecal carriers among the normal mice added as contacts.[4] The bacte-

[4] During the period covered by these experiments—December 17, 1924, to June 5, 1925,—about 3700 mice of our Institute strain were given out from the breeding

rial counts for the second half of the experimental period represent, therefore, a mixture of M.T. I and M.T. II, the relative proportions of which are unknown.

EXPERIMENTAL.

On December 17, 1924, twenty albino mice of our Institute strain each received *per os*, by stomach tube, 0.5 cc. of a 1:100 dilution of an 18 hour broth culture of M.T. II, each dose containing about 5,000,000 bacilli. After inoculation, 10 of the mice were placed in each of two wire-topped zinc boxes, measuring 5 × 7 × 10 inches, to which had been previously added 50 gm. of very fine sawdust. Immediately thereafter, and on each day throughout the period of observation, from December 17, 1924, to June 4, 1925, inclusive, two normal mice of our Institute strain were added to each box as contacts. As already described, the sawdust was removed from each box, and its M.T. II content was determined at regular intervals, every 2nd day in Box 1 and every 3rd day in Box 2. This had the effect of periodically modifying the available dosage of mouse typhoid bacilli in each box.

The results of these two experiments are shown in Text-fig. 1, the curves for Box 1 being on the left, those for Box 2 on the right. The construction of the curve showing the fluctuations in the number of mouse typhoid bacilli present in the sawdust of the box has already been described. The mortality curve was constructed by plotting the total number of mice dying in each successive 2 day period in Box 1, and in each successive 3 day period in Box 2. The curve of the total cage population was made by plotting the average number of mice present in the box for the 2 day period in Box 1, or the 3 day period in Box 2. The curve of survival time was constructed by plotting the average survival time of the group of four contact mice added to Box 1 in each successive 2 day period, or the group of six contact mice added to Box 2 in each successive 3 day period. Following the scheme employed by Topley,[5] all mice dead in less than 3 days after being added to the box have been omitted from the curve of survival time as representing deaths probably due to fighting. Similarly, all mice which survived beyond the experimental period have been eliminated from this curve, their presence in the group being indicated by an arrow to show that if the experiment had been prolonged the average survival time for the group would have been somewhat greater than that recorded.

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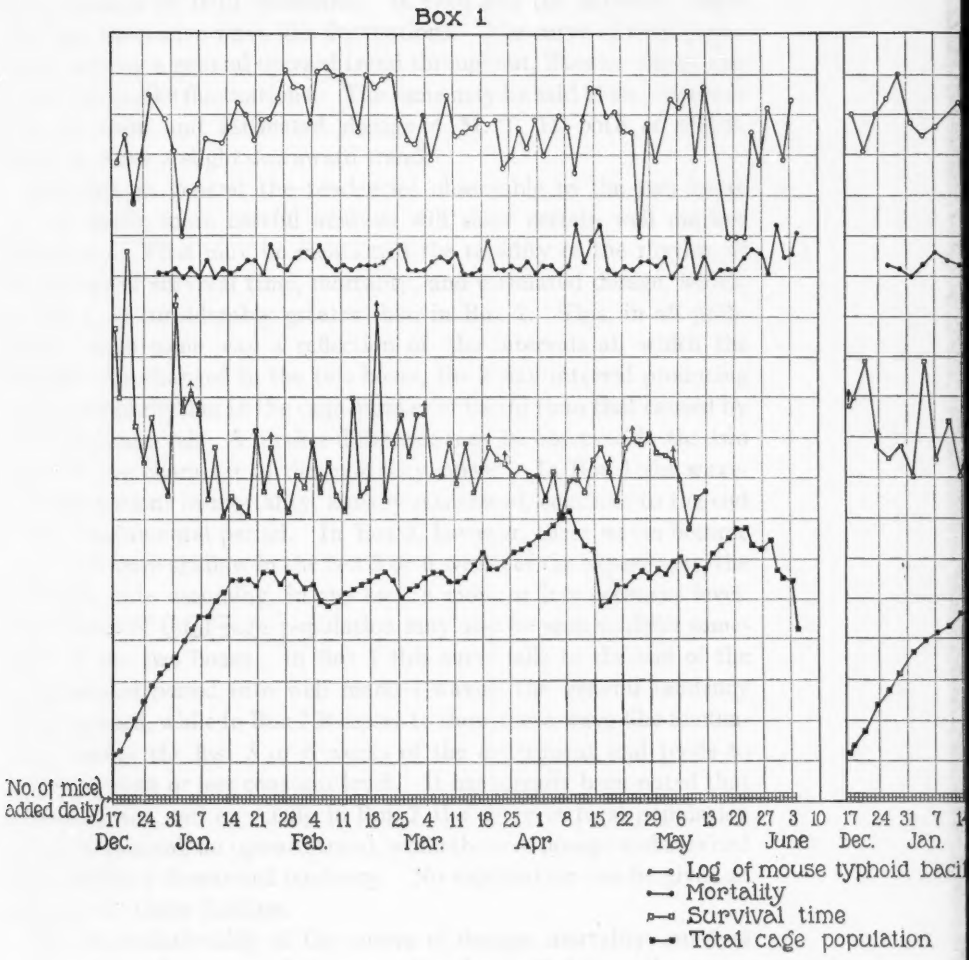
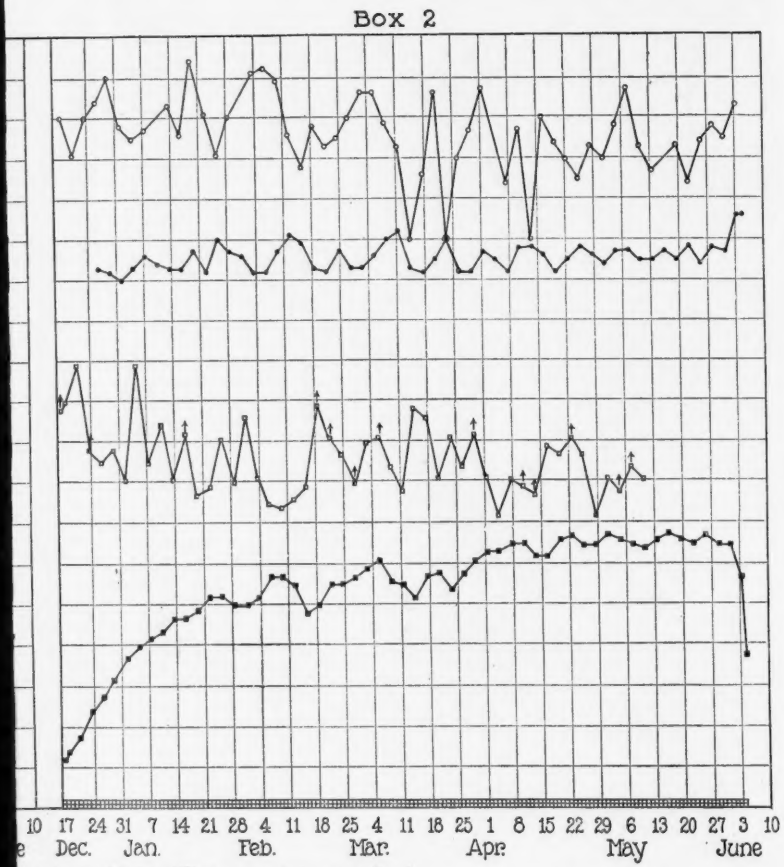
room. In addition, about 300 mice of this strain were constantly kept on hand there as breeders. During this same period, 9 adult mice of our Institute strain died in the breeding room, from causes not associated with pregnancy, and were autopsied. Of these, 3 yielded colonies of M.T. I (*Bacillus enteritidis*) from the internal organs or feces. It will be seen from the figures that the incidence of M.T. I in our breeding room, as indicated by the deaths due to this organism, is very small indeed.

[5] Topley, W. W. C., *J. Hyg.*, 1920–21, xix, 350.


Box 1
No. of mice added daily
17 24 31 7 14 21 28 4 11 18 25 4 11 18 25 1 8 15 22 29 6 13 20 27 3 10 17 24 31 7 14
Dec. Jan. Feb. Mar. Apr May June Dec. Jan.
Log of mouse typhoid bacil
Mortality
Survival time
Total cage population

TEXT-FIG. 1.


Box 2
10 17 24 31 7 14 21 28 4 11 18 25 4 11 18 25 1 8 15 22 29 6 13 20 27 3 10
e Dec. Jan. Feb. Mar. Apr. May June
se typhoid bacilli present in sawdust
time
e population

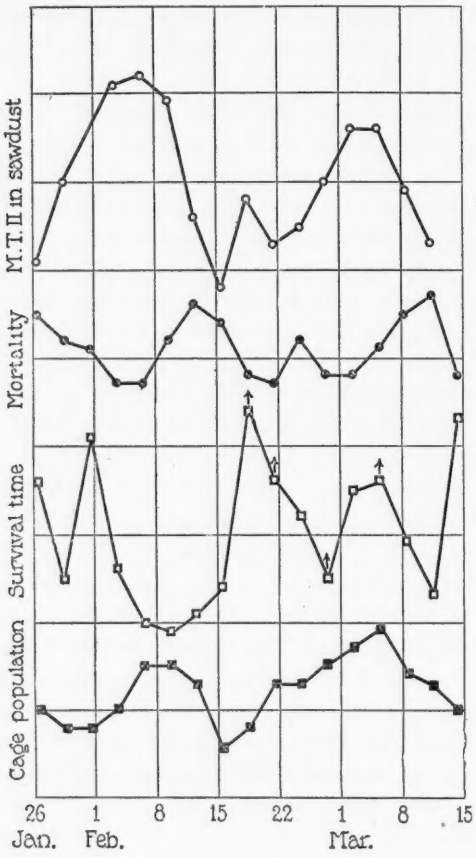
G. 1.

A study of the curves in Text-fig. 1 will show that certain phenomena were common to both epidemics. In each box the mortality curve falls into successive wave-like fluctuations. The curve of total population, having a general upward trend throughout, likewise shows successive wave-like fluctuations. The same may be said of the curves of survival time and estimated dosage of M. T. II, both of which, however, show a slight downward trend.

Although in general the tendencies observable in the two boxes are the same, more careful analysis will show certain well marked differences. First may be mentioned the rapidity of the rhythm of the curves of survival time, mortality, and estimated dosage, which, in Box 1, is considerably greater than in Box 2. This, in all probability, is in some way a reflection of the intervals at which the sawdust was changed in the two boxes, the 2 day interval producing a more rapid rhythm in the cage number of bacilli than that caused by the 3 day interval. A further difference may be observed in the two boxes in the character of the mortality curves. In Box 1 the wave-like fluctuations in mortality, already mentioned, continue to the end of the experimental period. In Box 2, however, these waves become more and more shallow in the last 5 or 6 weeks of the experiment, the mortality rate assuming, in the end, a more or less constant level. The curves of total cage population may also be seen to differ somewhat in the two boxes. In Box 1 this curve falls to the end of the experimental period into well marked waves, the general tendency being upward, while in Box 2 it ceases to show these wave-like fluctuations during the last 5 or 6 weeks of the experiment and tends to assume a more or less constant level. It has already been noted that in both boxes, and especially in Box 2, the curve of total population shows, in general, an upward trend, while those of dosage and survival time exhibit a downward tendency. No explanation can be given at present for these findings.

The interrelationship of the curves of dosage, mortality, survival time, and total cage population may best be studied from those portions of the curves of Box 2, included in the period January 26 to March 15, shown in Text-fig. 2. This particular period is chosen because of the wide rhythm of these curves. On January 26 the mortality curve had reached a peak, and in the days immediately fol-

lowing the mortality decreased, reaching a low point February 4 to 7. During this decline in the epidemic wave the number of mouse typhoid bacilli present in the sawdust was steadily rising, reaching its peak on



TEXT-FIG. 2.

February 7, at a time when the death rate was at a minimum. Immediately thereafter the mortality rate began to rise, reaching its peak 6 days after the peak in the bacterial count. The count continued to fall until February 16, after which there is recorded a slight

rise of very brief duration, which is, nevertheless, followed 6 days later by a small peak in the mortality curve. Again the bacterial count rose, reaching its maximum in the period March 3 to 6, and once more this peak is followed 6 days later by a peak in the mortality curve.

This recurrence of a 6 day interval between the period of maximum available dosage and the peak in the succeeding epidemic wave is, in our opinion, not a matter of chance. While it is shown most clearly in the period January 26 to March 15, it can be seen also in the two epidemic waves preceding January 26 (see Text-fig. 1, Box 2), so that in five successive epidemic waves, covering a period of 60 days, the same time relationship between high potential dosage and high mortality is found to hold good. Further significance is added to this fact, when we recall that in the many epidemics which we have initiated by inoculating *per os* the mice of our Institute strain with a known dose of mouse typhoid bacilli, the usual period of lag closely approximates this same interval. This is shown in the standard control curve constructed by Webster[6] for our Institute strain of mice, in which the period of lag is given as about 5 days, and in the similar curve reported for this same strain by Pritchett,[7] where the period of lag was found to be about 7 days.

A comparison of the curves of bacterial count and average survival time lends further emphasis to the relationship apparently existing between the number of bacteria present in the box and the mortality rate. It will be seen that as the bacterial count curve approaches its maximum, the survival time sharply declines, reaching its minimum at a point closely corresponding to the peak in the bacterial count. As the count falls again, the survival time rises sharply, to be again checked and brought down at points corresponding in general to peaks in the bacterial count.

The relationship of the population curve to the other curves, while not consistent throughout the experimental period, appears to be clear-cut in that portion of the chart shown in Text-fig. 2. It will be seen that the maxima in the total population curve closely correspond to the maxima in the bacterial count curve, while survival time

[6] Webster, L. T., *J. Exp. Med.*, 1923, xxxvii, 231.
[7] Pritchett, I. W., *J. Exp. Med.*, 1925, xli, 195.

tends to decrease with crowding. It is possible that crowding may lower resistance in such a way that more rapid multiplication of bacteria becomes possible in the host, leading to a greater output.

The points which stand out from a study of Text-fig. 2 are, briefly, these:

1. Under the conditions described, there seems to be a definite time relationship between maximum available dosage and maximum mortality. This relationship is reflected in the curve of survival time, the fluctuations of which are inversely proportional to the number of mouse typhoid bacilli present in the cage.

2. There seems to be some relationship between the size of the total population and the number of mouse typhoid bacilli present in the cage.

Determination of Virulence.

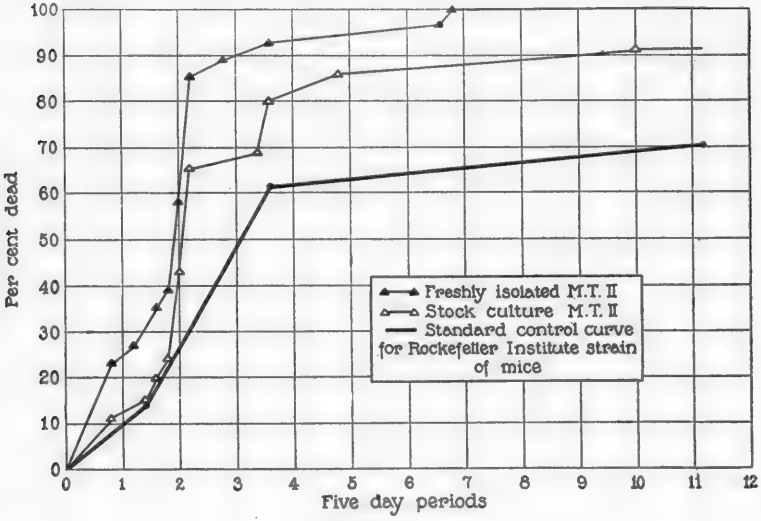
On May 29 there was isolated from the sawdust of Box 2 a culture of the original epidemic strain, M.T. II. At this time, and for about a month preceding it, the mortality rate of the mice in Box 2 had ceased to show the well marked periodic fluctuations so characteristic of the earlier part of the epidemic, and was assuming a relatively constant level, while at the same time the population curve was also losing its previous wave-like character and remaining approximately constant. A test was performed with this recently isolated passage strain of M.T. II to determine whether it possessed a virulence superior to that of the original stock strain with which the epidemic had been initiated. 26 mice of our Institute strain received *per os*, with a stomach tube, 0.5 cc. of an 18 hour broth culture of the freshly isolated organism (4,350,000 bacilli), and at the same time 27 control mice of the same strain received 0.5 cc. of an 18 hour broth culture of the original stock culture (4,150,000 bacilli). Text-fig. 3 shows the mortality curves for these two sets of mice, together with the standard control curve constructed for this strain of mice last year, which so closely approximates the similar curve constructed for this strain by Webster. It will be seen that there is very little difference in the response of the mice to these two organisms, one recently isolated after repeated animal passage in the course of an epidemic, the other a stock strain which is kept in the ice box on agar slants, and has received no animal passage for more than 3 years. Such difference as exists is chiefly one of lag, a variation of 9 per cent in the final mortality rates being of little significance with such small numbers of mice.

Autopsy of Survivors.

It seemed to us desirable to study the mice remaining alive in each box at the end of the period of observation, in order to determine what

percentage of them showed the presence of living organisms in their tissues, or of specific agglutinins in their blood serum.

On June 5 all the mice remaining alive in both boxes were autopsied. Each mouse was etherized, and while it was still breathing the abdomen and thorax were opened, care being taken not to sever any of the large blood vessels. With a small gauge needle and a 1 cc. Luer syringe as much blood as possible was removed from the right ventricle, while the heart continued to beat. A drop of this blood was placed on a 0.1 per cent glucose agar plate divided into quarters, and spread



TEXT-FIG. 3.

with a loop, the rest of the blood being placed in a slanting position in a sterile, plugged Wassermann tube and allowed to clot. Cultures were also made on the agar plate from the spleen, gall bladder, and feces, and the gross appearance of all these organs, and of the liver, was noted. Only 43 mice from Box 1 and 37 mice from Box 2 came to autopsy on June 5, because of the large number of deaths in both boxes due to the excessively high temperature prevailing in New York City during that week. The same effect was observed among the normal mice in our breeding room, large numbers of them succumbing to the heat during this period.

The pathological, serological, and cultural findings from all these mice are given in Tables I and II.

It will be seen from a study of Tables I and II that the vast majority

TABLE I.

Box 1.		Gross pathology.				Suspicious colonies.				Mouse typhoid bacilli. Serological types isolated.				Titre blood agglutinins.	
Mouse.	Days in box.	Liver.	Spleen.	Gall bladder.	Feces.	Heart's blood.	Spleen.	Feces.	Gall bladder.	Heart's blood.	Spleen	Feces.	Gall bladder.	M.T. I.	M.T. II.
16	155	Normal.	±	+	±	−	+	+	+		I	−	−	−	−
17	154	"	Normal.	Normal.	Normal.	−	−	+	−			−		640	−
19	152	"	"	"	"	−	+	+	−		I	−		40	−
39	132	"	"	"	"	−	+?	+?	−		I	−		−	−
51	119	"	"	"	"	−	+	+	+		I	−	I	−	−
66	105	+	+	+	±	−	−	+	−			−		−	−
78	93	Normal.	Normal.	Normal.	Normal.	−	+	−	−		I			−	−
93	78	"	"	"	"	−	−	+?	−			−		−	−
98	73	+	"	±	+?	−	−	+	+			−	−	−	−
116	55	Normal.	"	Normal.	Normal.	−	−	−	−	−	−	−	−	−	−
123	48	"	"	"	"	−	−	+?	−			−		160	−
127	44	±	±	+?	"	+	+	−	+	I	I	−	I	−	−
129	42	Normal.	Normal.	Normal.	"	+	+	+	−	I	I	−		−	−
135	36	"	"	"	"	+	+	+	−	I	I	−		−	−
138	33	"	"	"	"	−	−	+?	−			−		−	−
139	32	"	"	"	"	−	−	+?	−			−		−	−
143	28	"	"	"	"	−	+?	+?	+?		I	−	I	−	−
149 A	22	"	"	"	"	−	−	+?	−			−		−	−
149 B	22	"	"	"	"	−	−	+?	−			−		−	−
150	21	"	"	"	"	−	+	+?	−		I	−		−	−
151	20	"	"	±	"	+	+	+	+	I	I	I	I	−	−
153 A	18	+	+	Normal.	"	+	+	−	+	I	I	−	I	−	−
153 B	18	Normal.	Normal.	"	"	−	−	−	−					−	−
154	17	"	"	"	"	−	−	+	−			−		−	−

156	15	Normal.	Normal.	Normal.	Normal.	+	+	−	+	I	I		I	−	−	−
157 A	14	"	"	"	"	−	−	−	−					−	−	−
157 B	14	"	"	"	"	+*	+	+*	+	I	I	I	I	−	−	−
159 A	12	"	"	"	"	−	−	+?	−			−		−	−	−
159 B	12	"	"	"	"	−	−	−	−					−	−	−
160	11	"	"	+?	"	−	−	+?	−			−		−	−	−
161 A	10	"	"	Normal.	"	−	−	+	−	I	I	−	I	−	−	−
161 B	10	"	"	+?	"	+	+	+	+	I	I	I	I	−	−	−
162	9	"	"	Normal.	"	−	−	−	−					−	−	−
163	8	"	"	"	"	−	+	+	−		I	−		−	−	−
164	7	"	"	"	"	−	−	−	−					−	−	−
165 A	6	"	"	"	"	−	−	+	+			−		−	−	−
165 B	6	"	"	"	"	−	+	+	−		−	−		−	−	−
166 A	5	"	"	"	"	+*	−	−	−	I				−	−	−
166 B	5	"	"	"	"	−	−	+	−			I		−	−	−
167	4	"	"	"	"	−	−	+	−			−		−	−	−
169	2	"	"	"	"	−	−	+	−			−		−	−	−
170 A	1	"	"	"	"	−	−	−	−					−	−	−
170 B	1	"	"	"	"	−	−	+?	−			−		−	−	−

* Mucoid colonies only.

TABLE II.

Box 2.		Gross pathology.				Suspicious colonies				Mouse typhoid bacilli. Serological types isolated.				Titre blood agglutinins.	
Mouse.	Days in box.	Liver	Spleen.	Gall bladder.	Feces.	Heart's blood.	Spleen.	Feces.	Gall bladder.	Heart's blood.	Spleen.	Feces.	Gall bladder.	M.T. I.	M.T. II.
Inf.*	170	Normal.	Normal.	Normal.	Normal.	−	−	+?	+?			−	−	−	−
7	164	"	"	"	"	−	−	+?	−			−		−	−
25	146	"	"	"	"	−	+	+?	−		II	−		−	−
30	141	"	"	"	"	−	−	+?	−			−		−	−
63	108	"	"	"	"	−	−	+?	−			−		−	−
68	103	"	"	"	"	−	−	+?	−			−		−	−
74	97	"	"	"	"	−	+	+?	−		I	−		−	−
78	93	"	"	±	"	−	−	+	−			I		−	−
79	92	"	"	Normal.	"	−	−	−	−					−	−
103	68	"	"	"	"	−	+?	−	−		II			−	−
114	57	"	"	"	"	−	−	+?	−			−		−	−
117	54	"	"	"	"	−	−	+	−			−		−	−
128	43	"	"	"	"	−	−	+?	−			−		−	−
139	32	"	"	"	"	−	−	−	−					−	−
140	31	"	"	"	"	+	+	−	+	I	I		I	−	−
142	29	"	"	"	"	−	−	+?	−			−		−	−
143	28	"	"	"	"	+†	+†	+†	+†	I	I	I	I	−	−
148	23	"	"	"	"	−	−	−	−					−	−
149 A	22	"	"	"	"	+†	+†	−	−	I	I			−	−
149 B	22	"	"	"	"	−	−	+?	−			−		−	−
154	17	"	"	"	"	−	−	+?	−			−		−	−
156	15	"	"	"	"	+	+	+	+	I	I	−	I	−	−
158 A	13	"	"	"	"	−	−	+?	−			I		−	−
158 B	13	"	"	"	"	−	−	+?	−			−		−	−

161 A	10	Normal.	Normal.	Normal.	Normal.	−	−	+	−			−		−	−
161 B	10	"	"	"	"	+	+	+	−	−	I	−		−	−
162 A	9	"	"	"	"	−	+	+	+		I	−	I	−	−
162 B	9	"	"	"	"	−	−	−	−					−	−
163 A	8	"	"	"	"	−	−	+	−			−		−	−
163 B	8	"	"	+?	"	−	−	+?	+?			−	−	−	−
164	7	"	"	Normal.	"	−	−	+	+			−	−	−	−
165	6	"	"	"	"	−	−	+?	−			−		−	−
166 A	5	"	"	"	"	−	−	−	−					−	−
166 B	5	"	"	"	"	−	−	+	−			−		−	−
167 A	4	"	"	"	"	−	−	−	−					−	−
167 B	4	"	"	"	"	−	+	+	−		I	−		−	−
169	2	"	"	"	"	−	−	+	+			−	−	−	−

* One of the mice originally infected *per os* by stomach tube.

† Mucoid colonies also found.

of the mice in both boxes, coming to autopsy on June 5, showed no gross indications of having been infected with the bacillus of mouse typhoid. As is indicated by plus marks, an occasional liver showed slight scarring, the spleen was sometimes slightly swollen or scarred, a few gall bladders were whitish in appearance, and in a very few mice the feces were perhaps faintly tinged with blood. Nevertheless, mouse typhoid bacilli, identified by agglutination tests, were isolated from the heart's blood, feces, and internal organs in a fairly high percentage of cases (Table III).

The predominating organism isolated from these mice was not, however, the organism with which the epidemic was initiated (M.T. II), but rather the M.T. I which probably gained entrance to the boxes,

TABLE III.

	Surviving mice positive for mouse typhoid bacilli.								Total positive.	
	Heart's blood.		Spleen.		Feces.		Gall bladder.			
	M.T. I.	M.T. II.	M.T. I.	M.T. II.	M.T. I.	M.T. II.	M.T. I.	M.T II.	M.T. I.	M.T. II.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Box 1.....	21	0	37	0	9	0	19	0	42	0
Box 2.....	11	0	22	5	8	0	11	0	27	5

as already indicated, through the presence of healthy carriers among the mice added as contacts. In Box 1 the original M.T. II had apparently been completely eliminated, while in Box 2 it was found in only 5 per cent of the mice examined, each time in the spleen. Yet it was probably still present in small numbers even at this time, in both boxes, since Type II organisms were found, together with Type I, in Mouse 138, Box 1, which died June 2, after living 30 days in the box, while both types were recovered from the sawdust of Box 1 as late as April 13. In Box 2 both types were recovered from Mouse 157, dying June 5, after living 14 days in the box, and Type II, together with Type I was recovered from the sawdust of the box as late as May 29.

DISCUSSION.

Topley, in his early studies of mouse typhoid epidemics, attributed the rise and fall and periodic recurrence of epidemic waves to fluctuations in the virulence of the microbic incitant.[8] On this basis also he explained the fact that the duration of life of additions made to a population in the early stages of an epidemic was considerably shorter than when they were introduced after the period of maximum mortality had passed.[5] Webster, on the contrary, could find no significant fluctuations in the pathogenicity of mouse typhoid bacilli,[9] and from the evidence gained through an experimental analysis of virulence, microbic dosage, and host susceptibility believed that the phenomena of recurrent epidemic waves arise from fluctuations in the two latter factors.

The experiments described in this paper were planned to test these opposing points of view under conditions as natural and as carefully controlled as may be. Shortly before they were begun, Topley and his coworkers published the results of a study of the number of mouse typhoid bacilli excreted from day to day by each member of an infected mouse population. They found that the excretion rate of the various individuals composing the group rose simultaneously before a rise in mortality rate, and declined at the time of its fall.[10] In a more recent paper on mouse pasteurellosis, Greenwood and Topley[11] showed that when larger numbers of normal mice were added daily to an infected population the resulting mortality curve tended to assume a high general level, with relatively slight fluctuations and minimal interepidemic periods. As the number of daily additions was decreased, the height and duration of the successive epidemic waves was increased, and the interepidemic periods became well marked and of considerable extent. In our experiments, the mortality curve with slight and rapid fluctuations occurred in the community where the dosage was affected arbitrarily by changing the sawdust at short intervals, while in the community whose mortality curve showed ample waves and well

[8] Topley, W. W. C., *Lancet*, 1919, ii, 1.
[9] Webster, L. T., *J. Exp. Med.*, 1923, xxxvii, 781.
[10] Topley, W. W. C., Ayrton, J., and Lewis, E. R., *J. Hyg.*, 1924–25, xxiii, 223.
[11] Greenwood, M., and Topley, W. W. C., *J. Hyg.*, 1925, xxiv, 45.

marked interepidemic periods the interval at which dosage was arbitrarily modified was longer. Hence by affecting the rate of immigration, or by varying the available dosage directly, one can modify at will the character of the epidemic waves and the interepidemic period. Thus our observations agree with the recent ones of Topley and his coworkers and, taken together with them, constitute strong evidence of the view put forward by Webster,[3] concerning the importance of dosage.

One further point in the resistance of the survivors of an epidemic deserves emphasis at this time. Webster has pointed out that a certain percentage of mice, given an infecting dose of mouse typhoid bacilli *per os*, shows no signs of infection,[6] and he later showed that when survivors were exposed a second time to a similar infection it was this so called natural resistance as much as any specific acquired immunity which again protected them.[12] Topley, however, in his examination of survivors, has been able to cultivate the bacilli from the internal organs in most instances, and he inclines to the view that specific acquired immunity, and perhaps a type of depression immunity similar to that studied by Lange[13] and by Morgenroth,[14] are responsible for the enduring resistance. The survivors in our own experimental series are of interest, when examined from this point of view, since they show a large percentage of naturally resistant individuals free from all signs of infection.

SUMMARY.

Epidemics of mouse typhoid set up among the Rockefeller Institute strain of mice were studied over a period of 6 months. During this time the relationship of cage number of mouse typhoid bacilli to mortality, total population, and survival time was determined. A single virulence titration of the epidemic strain was made, and at the end of the experiment all survivors were examined for evidence of infection. The following conclusions may be drawn for the data here presented.

[12] Webster, L. T., *J. Exp. Med.*, 1924, xxxix, 129.

[13] Lange, B., *Z. Hyg. u. Infectionskrankh.*, 1921, xciv, 135; 1924, cii, 224.

[14] Morgenroth, J., Biberstein, H., and Schnitzer, R., *Deutsch. med. Woch.*, 1920, xlvi, 337.

1. The available dosage of mouse typhoid bacilli varied directly with the mortality (plus a time constant of 6 to 8 days) and inversely with the survival time.

2. The virulence of the epidemic strain appeared to be practically the same as that of the original stock culture.

3. About 53.5 per cent of the survivors of one epidemic and 68 per cent of those in the other showed, at the end of the experiment, no signs of infection; the others had either specific blood agglutinins, or living bacteria in their heart's blood, spleen, feces, or gall bladder.

4. During the course of the epidemic, the original infecting strain (mouse typhoid Type II—*Bacillus pestis caviæ*) was almost entirely replaced by an antigenically dissimilar strain (mouse typhoid Type I—*Bacillus enteritidis*), probably introduced through the inadvertent inclusion of fecal carriers among the normal mice added as contacts.

MICROBIC VIRULENCE AND HOST SUSCEPTIBILITY IN PARATYPHOID-ENTERITIDIS INFECTION OF WHITE MICE.

X. The Relative Susceptibility of Different Strains of Mice to Per Os Infection with the Type II Bacillus of Mouse Typhoid (Bacillus pestis caviæ).

Further Studies.

By IDA W. PRITCHETT, Sc.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, October 22, 1925.)

In a previous paper[1] we have compared the relative susceptibility of five different strains of mice to *per os* inoculation with a standard dose of mouse typhoid bacilli, Type II (about 5,000,000 organisms). Experiments have been continued through another year with four of these strains, two white and two colored, in which the following numbers of each strain were used.

Bagg mice (albino)	576
Lathrop mice (black)	631
Little mice (dilute brown)	313
Rockefeller Institute mice (albino)	580
Total	2100

The histories of these mice, as far as they are known to us, have already been recorded.[1]

EXPERIMENTAL.

As in the preceding year, monthly tests were run with all these strains of mice, beginning in October, 1924, so that the entire series

[1] Pritchett, I. W., *J. Exp. Med.*, 1925, xli, 195.

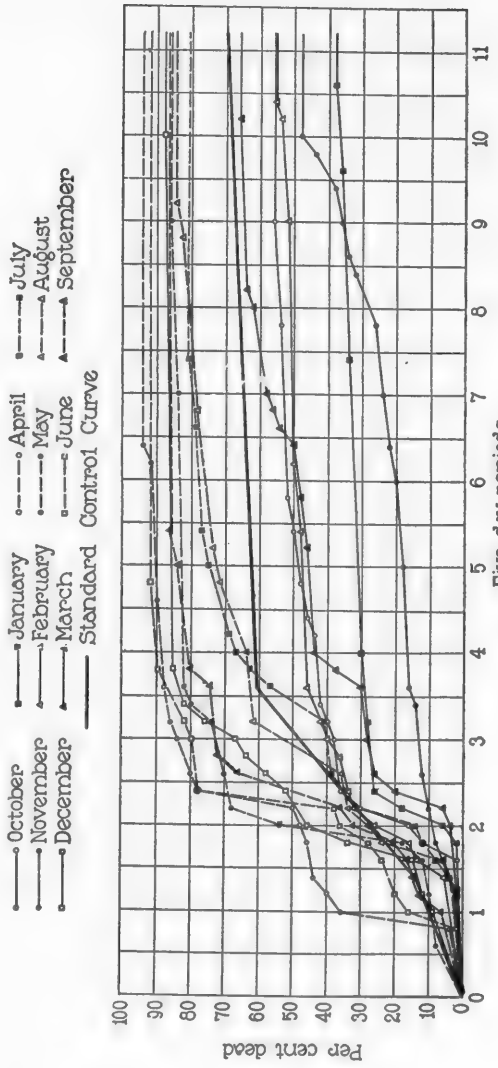
of experiments, for both years, covers a period of 24 consecutive months, from October, 1923, to September, 1925 inclusive. An effort was made to have at least 50 mice of each strain for each monthly test, but in some instances the number fell below this standard, especially with the Little mice. The total number of mice, therefore, was only 2100, as compared to a total of 2540 mice of these four strains in the previous year. All strains were strictly strain-inbred.

The routine procedure in starting one of these monthly tests was as follows:

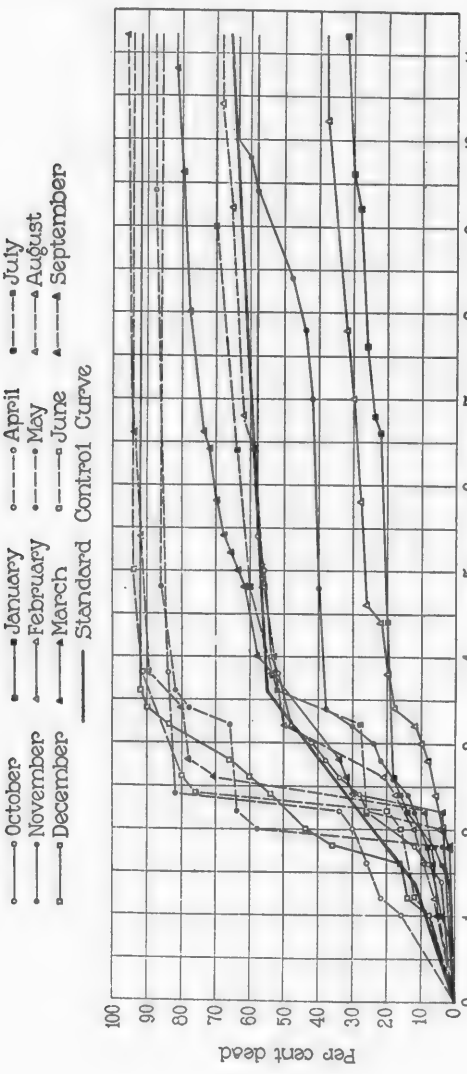
> The mice of all strains were brought from the breeding room and assembled on the day before inoculation, each mouse in a separate battery jar partly filled with shavings. The next morning each mouse received *per os*, with a stomach tube, 0.5 cc. of an 18 hour broth culture of M.T. II, diluted 1:100, this dose containing usually about 5,000,000 organisms. All animals were fed directly after the administration of the infecting dose with the usual daily diet of bread soaked in pasteurized Grade B milk. At about 9 o'clock each morning the deaths for the preceding 24 hours were recorded. No autopsies were performed on any of these mice, since our previous experience had indicated that only a very small percentage of our deaths was due to non-specific causes. All experiments were allowed to run 8 weeks.

In Text-figs. 1 to 4 are shown all the twelve curves plotted for each of the four strains of mice, in the course of the 12 month period. These charts may be compared with the corresponding charts in the paper already referred to,[1] where the curves for the previous 12 month period are shown. Text-fig. 1 gives the twelve curves for the Rockefeller Institute mice. It will be seen from this chart, as well as from Text-figs. 2, 3, and 4, which show the curves for the Bagg, Lathrop, and Little mice respectively, that the scatter in the various monthly curves is rather more than that recorded for these same strains in the preceding year. The Lathrop mice, however (Text-fig. 3), remained the most constant in their response to the infection, showing about the same degree of scatter that they showed in the previous year. A comparison of the scatter shown by the different strains for the 2 years is given in Table I.

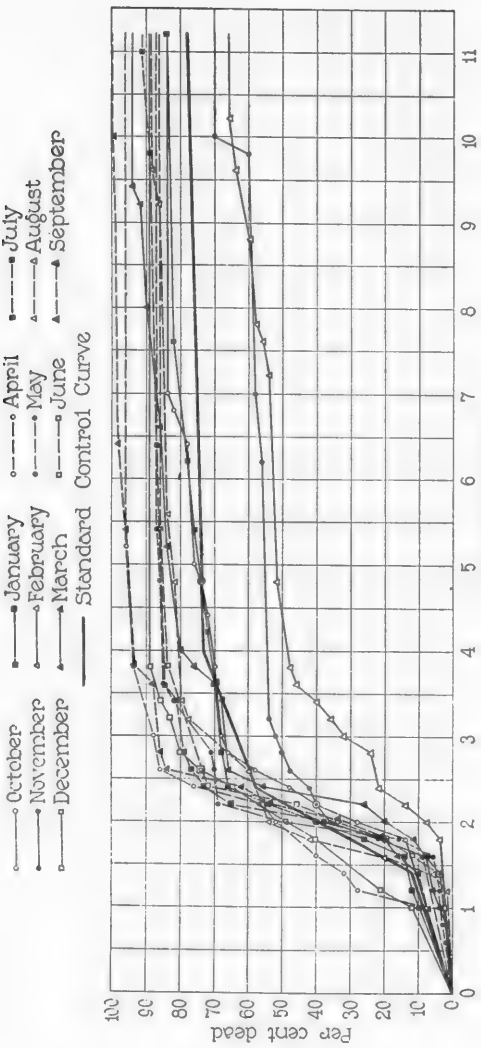
Although the degree of scatter is slightly greater this year than last, the various strains retain the same relative standing with respect to each other. The Lathrops and Littles, both colored strains, show still a greater degree of constancy in their response to the infection, as



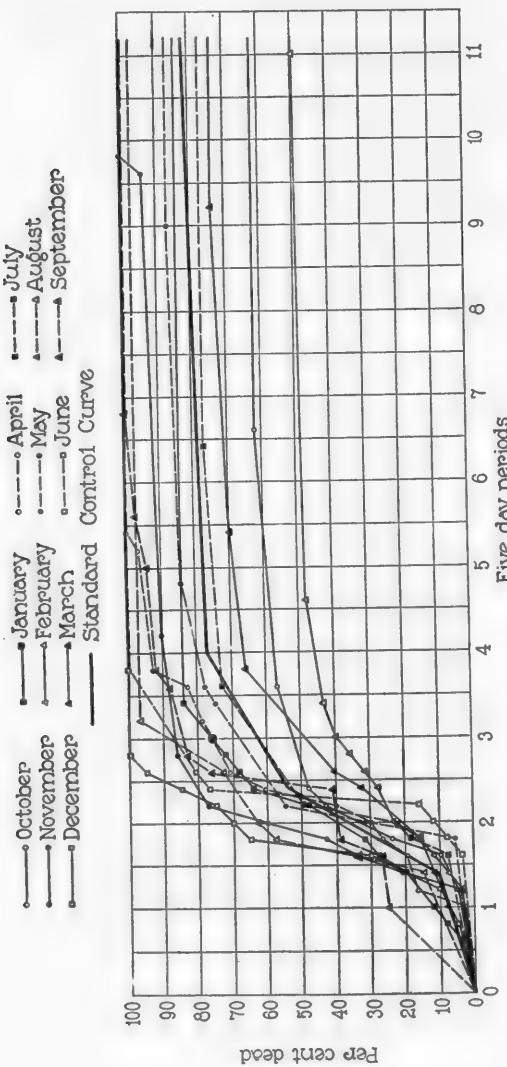
TEXT-FIG. 1. Rockefeller Institute mice.



TEXT-FIG. 2. Bagg mice.



TEXT-FIG. 3. Lathrop mice.



TEXT-FIG. 4. Little mice.

indicated by the degree of scatter in their curves, than do the albino strains. The seasonal variation in the susceptibility of the different strains, which is the source of the scatter in the curves, will be discussed in another paper.[2]

In Text-fig. 5 are shown average curves constructed for each strain of mice from all the curves for that strain in the course of the 12 month period, together with the standard control curve obtained last year for the Rockefeller Institute strain. Reference to the earlier paper,[1] and to Text-fig. 6 of the present paper will show that these average curves closely approximate, both in height and pitch, the similar curves obtained for these four strains of mice last year. Again the average mortality is lower for the albino strains (Bagg and Rockefeller Institute), the two dark strains (Lathrop and Little) showing a final

TABLE I.

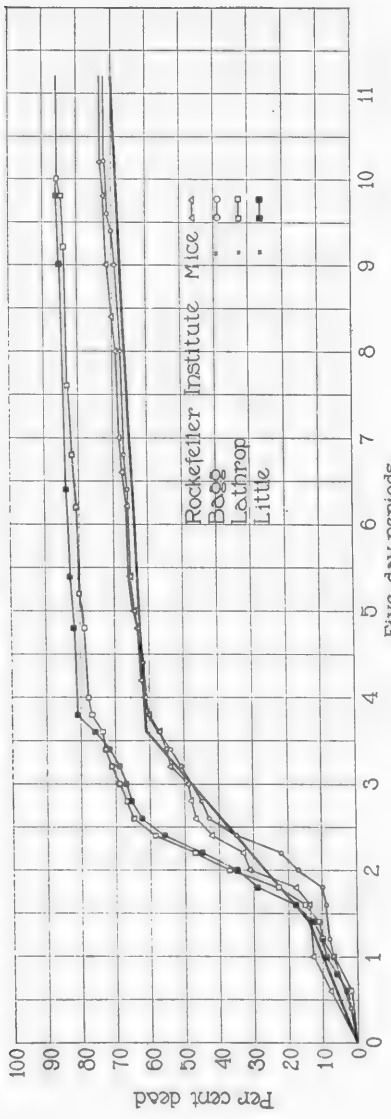
Strain.	Difference separating lowest and highest mortality rates.	
	per cent, 1923–24	*per cent, 1924–25*
Lathrop mice (black)	30	34
Little " (dilute brown)	34	50
Institute " (albino)	50	56
Bagg " (albino)	55	65

mortality about 13 per cent above that of their white companions. As was also observed last year, the pitch of the curves for the colored strains is steeper than that of the curves for the white strains. In short, for all strains much the same relative susceptibility may be seen to exist that was recorded in the previous 12 month period.

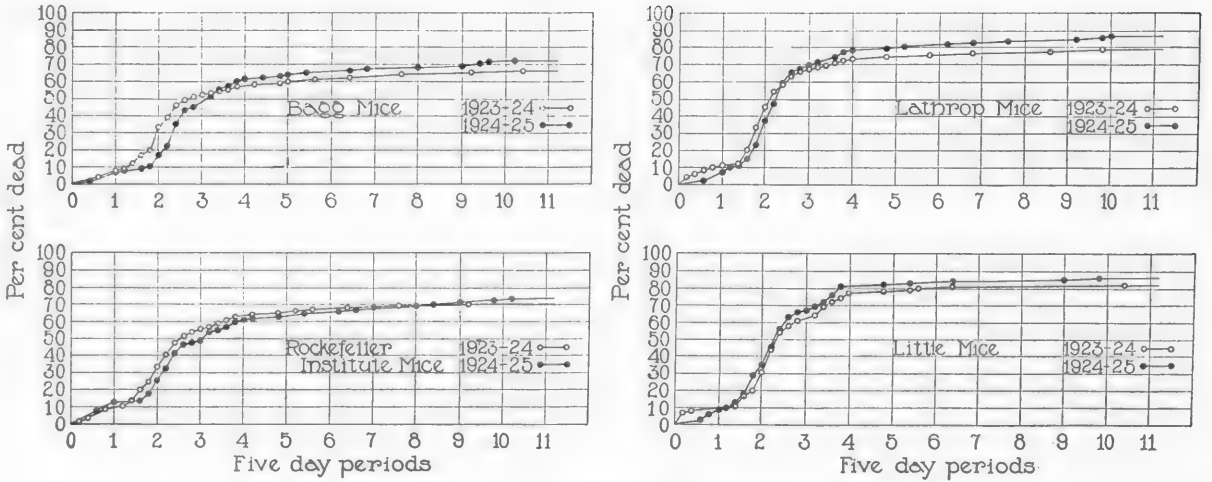
In Text-fig. 6 are shown the average curves constructed for each of the four strains of mice this year and last. It will be seen how closely the curves obtained in the second year approximate those for the preceding 12 month period, the final mortality rate for all strains being slightly higher.

In Text-fig. 7 are shown standard control curves constructed for each strain from all the mice used in these experiments to date. The standard control curve for the Rockefeller Institute strain has been

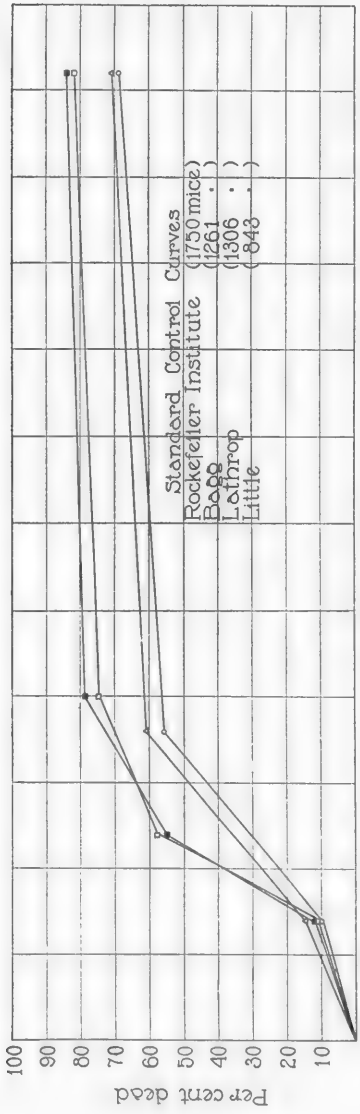
[2] Pritchett, I. W., *J. Exp. Med.*, 1926, xliii, 173.


Per cent dead
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
9
10
11
Five day periods
Rockefeller Institute Mice
Bogg
Lathrop
Little

TEXT-FIG. 5.


Per cent dead
Bagg Mice
1923-24
1924-25
Lathrop Mice
Rockefeller Institute Mice
Little Mice
Five day periods

TEXT-FIG. 6.


Standard Control Curves
Rockefeller Institute (1750 mice)
Bage (1261 ")
Lathrop (1306 ")
Little (843 ")
Per cent dead
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
Five day periods

TEXT-FIG. 7.

constructed by averaging the two curves obtained for this strain in the past 2 years, together with the standard control curve originally constructed for this strain by Webster,[3] which the two later curves so closely approximate. The various standard control curves given in Text-fig. 7 are based on the following numbers of mice.

Bagg (albino)	1261 mice.
Lathrop (black)	1306 "
Little (dilute brown)	843 "
Rockefeller Institute (albino)	1750 "

The large numbers of mice of known age and parentage employed in these experiments; the repeated monthly tests over a period of 2 years, with all conditions as nearly as possible the same; the characteristic mortality curve of each strain, varying from month to month in a more or less predictable manner, and yet retaining a constant mean; the same consistent differences between the races—all these things show clearly that these strains of mice inherit, from generation to generation, a definite amount of resistance, affected by external influences, but under constant conditions varying among different individuals about a mean which is characteristic of the race.

Further evidence for this belief has been brought forward by Webster, who has shown that selective breeding of the most resistant or most susceptible individuals in a given race transmits a greater or less resistance to succeeding generations.[3,4]

SUMMARY.

Four separate strains of mice have been tested for their relative susceptibility to *per os* infection with the Type II bacillus of mouse typhoid (*Bacillus pestis caviæ*), 300 to 600 individuals of each strain having been employed in the course of 12 months. In confirmation of a previous paper, clear-cut differences in the susceptibility of these strains have been shown to exist. In general, the colored strains were distinctly less resistant than the albino strains.

[3] Webster, L. T., *J. Exp. Med.*, 1924, xxxix, 879.
[4] Webster, L. T., *J. Exp. Med.*, 1925, xlii, 1.

MICROBIC VIRULENCE AND HOST SUSCEPTIBILITY IN PARATYPHOID-ENTERITIDIS INFECTION OF WHITE MICE.

XI. Seasonal Variation in the Susceptibility of Different Strains of Mice to Per Os Infection with the Type II Bacillus of Mouse Typhoid (Bacillus pestis caviæ).

Further Studies.

By IDA W. PRITCHETT, Sc.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, October 22, 1925.)

In a recently published paper[1] we have drawn attention to the fact that a marked seasonal variation in susceptibility to infection with the bacillus of mouse typhoid has been observed with five unrelated strains of mice, all fed on the same food and subjected to the same living conditions. The experiments previously reported were begun in October, 1923, and those to be discussed now, performed with four of the five strains, followed directly the ones of the previous year. Thus we have a record of the response of these four strains of mice to a standard dose of the bacillus of mouse typhoid from October, 1923, to September, 1925, inclusive, the only gaps in the record being for the Lathrop strain in November, and for the Little strain in October and November of 1923.

EXPERIMENTAL.

The material presented here is the same as that employed in another paper on the relative resistance of different strains of mice to mouse typhoid,[2] the Bagg, Lathrop, Little, and Rockefeller Institute strains

[1] Pritchett, I. W., *J. Exp. Med.*, 1925, xli, 209.

[2] Pritchett, I. W., *J. Exp. Med.*, 1926, xliii, 161.

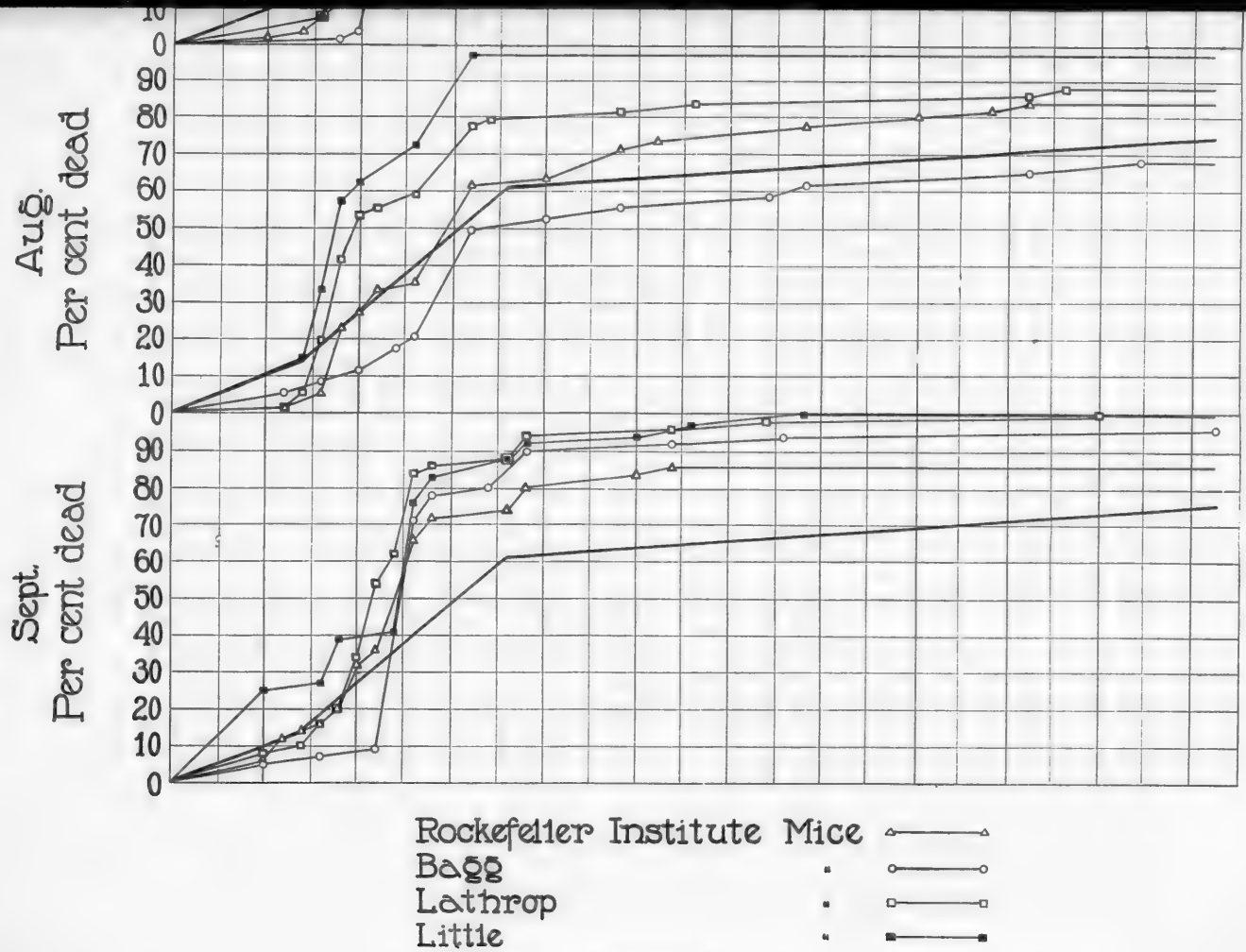
being used for these tests. The histories of these strains are given elsewhere.[3]

As in the preceding year, routine monthly tests were made with all four strains of mice for the period from October to September inclusive. The procedure employed in starting one of these monthly tests was the same as that used in the earlier work. On the day before inoculation with the mouse typhoid bacillus, each mouse was placed in a separate battery jar partly filled with shavings. The next morning a dilution of 1:100 of an 18 hour broth culture of M. T. II was made in bouillon, from which three dilution plates were poured for the purpose of counting the organisms present. Each mouse then received by stomach tube 0.5 cc. of this 1:100 dilution of the original culture, containing on an average about 5,000,000 mouse typhoid bacilli. The whole series of mice was fed directly after the inoculation with the usual daily diet of bread soaked in pasteurized Grade B milk. Each test was allowed to run for 8 weeks, during which time the deaths were recorded daily and mortality curves were plotted.

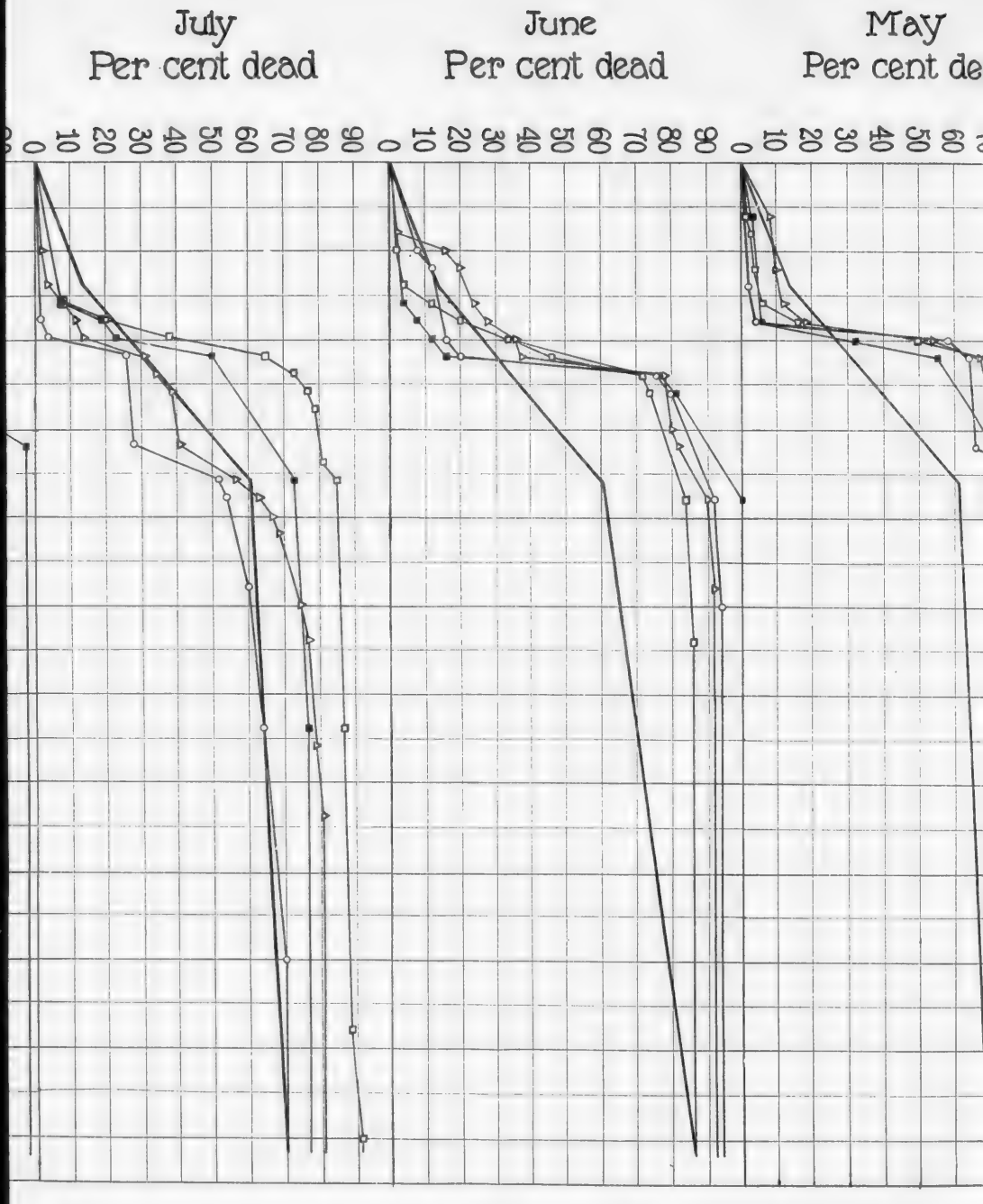
In Text-fig. 1 are shown the results of the twelve monthly tests. The charts are arranged in chronological order, from above downward, and in each chart is included in addition to the curves for the four strains of mice, the standard control curve constructed last year for our Institute albino strain, which closely approximates the similar curve previously constructed by Webster[4] for this strain; this control curve, since it remains constant throughout, is useful as a graphic indicator of the rise and fall of the other curves in the course of the 12 month period. This chart may be compared with Text-fig. 1 of the paper already referred to,[1] having been constructed in precisely the same manner.

Comparison of these charts with the corresponding charts of the previous year will show certain differences in the response of the mice to mouse typhoid in the two periods. The seasonal fluctuation in resistance during the first year (1923–24) followed a fairly regular course—a low mortality rate during the fall and winter, rising to a high peak in the spring (February to May inclusive); a very low level during the summer months of June and July, followed by another peak in September. In the year just past (1924–25) this same sequence of events is not quite maintained. The first variation from the standard established in the 1st year is the surprisingly high set

[3] Pritchett, I. W., *J. Exp. Med.*, 1925, xli, 195.
[4] Webster, L. T., *J. Exp. Med.*, 1923, xxxvii, 231.


Aug.
Per cent dead
Sept.
Per cent dead
0
10
20
30
40
50
60
70
80
90
Rockefeller Institute Mice
Bagg
Lathrop
Little

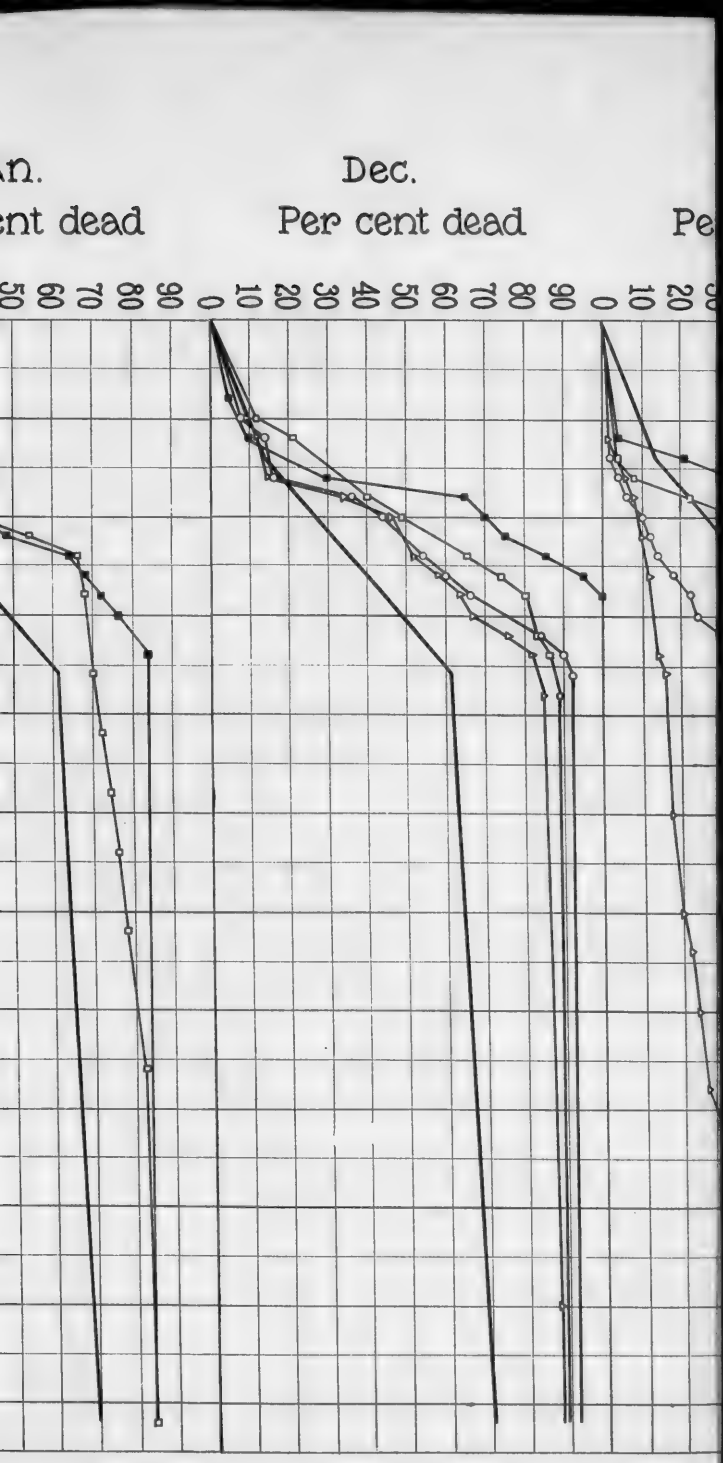
TEXT-FIG. 1.


July
Per cent dead
0 10 20 30 40 50 60 70 80 90
June
Per cent dead
0 10 20 30 40 50 60 70 80 90
May
Per cent de
0 10 20 30 40 50 60

Apr.
Per cent dead

Mar.
Per cent dead

Mar.
Per cent dead
Feb.
Per cent dead
0 10 20 30 40 50 60 70 80 90 100
0 1 2 3 4 5 6 7 8 9 10 11


an.
ent dead
50 60 70 80 90
Dec.
Per cent dead
0 10 20 30 40 50 60 70 80 90
Pe
0 10 20

Nov.
Per cent dead
0 10 20 30 40 50 60 70 80 90
Oct.
Per cent dead
0 10 20 30 40 50 60 70 80 90 100
0 1 2 3 4 5 6 7 8 9 10 11

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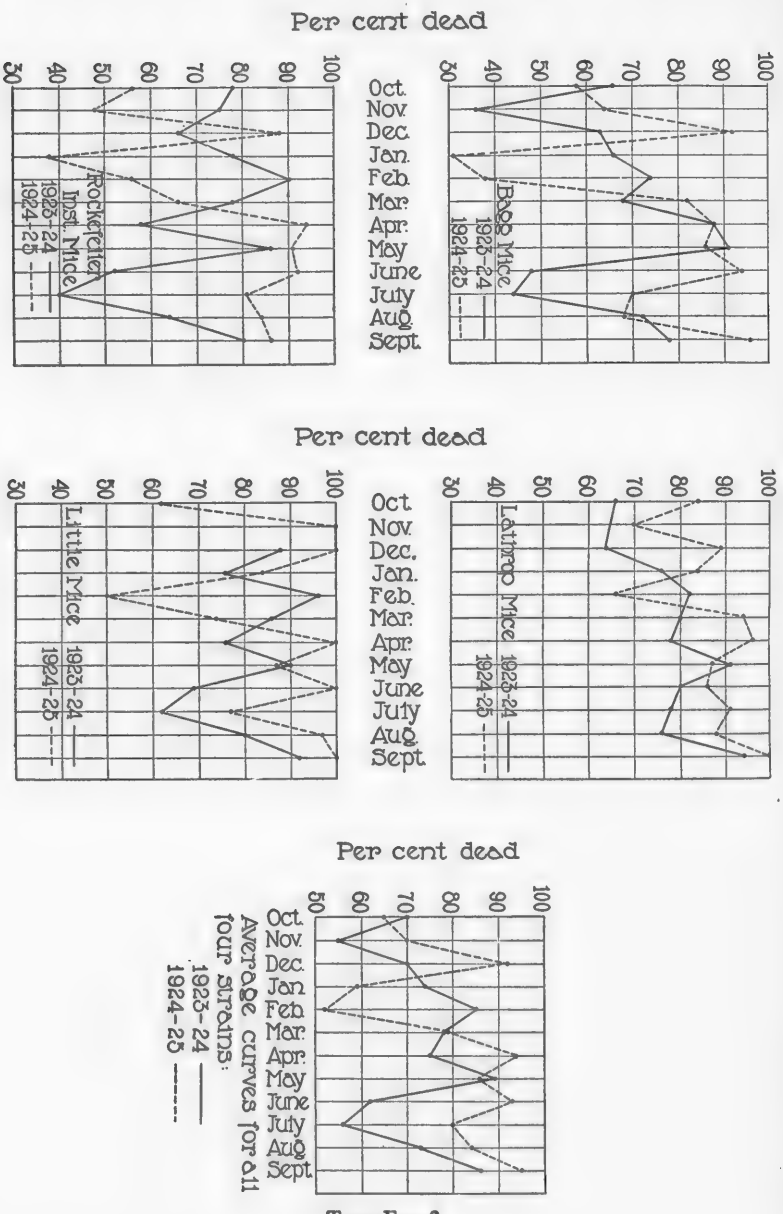
of curves obtained in December, comparable to those seen in the preceding spring months. This peak is followed by two sets of low curves, comparable to those seen in the preceding summer. In March, however, an upward tendency becomes apparent in all strains and the next 3 months—April to June inclusive—show the same high mortality rate that was so striking in the spring months of the year before. As in the preceding year, the July curves were, in general, the lowest for the summer, the Lathrop mice alone showing a lower summer mortality rate in June. Starting with the month of August there is again an upward trend in the curves, except for the Bagg curve, which drops still lower in August. In September all curves are high, their pitch being especially steep.

In Text-fig. 2 the seasonal variation in susceptibility of all strains of mice for the 2 years may be easily compared. In this chart appear the curves constructed last year by plotting for each strain of mice the total mortality occurring in each successive monthly experiment, the mortality figure given in any one month representing the mortality rate for the experiment begun in that month. (These same curves may be found in Text-fig. 2 of the paper already referred to.[1]) Superimposed on these curves are similar curves constructed from the total mortality figures obtained in the monthly experiments of the past year. Average curves for the four strains of mice for the 2 years are also included.

It will be seen that, in general, the same thing has happened in all strains in each of the 12 month periods. There has been a high mortality rate during the spring, a lower summer rate, and a subsequent fall rise. During the winter of 1924–25, however, there seems to have been a secondary rise in the mortality rate of all strains in December, followed by a lower rate in January, and an extraordinarily low rate in February, before the upward trend begins toward the spring peak.

No explanation for the departure from the record of the previous winter can be offered now. Circumstances of an undetermined nature obviously produced a marked simultaneous effect on all the four strains of mice in December, 1924, through which mortality was increased, just as other circumstances, equally unknown, brought about a lowering of the mortality rate during the 2 months following.


Per cent dead
30 40 50 60 70 80 90 100
Rockefeller Inst. Mice
1923-24
1924-25
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.
Sept.
Bagg Mice
1923-24
1924-25
Per cent dead
Little Mice 1923-24 1924-25
Latham Mice 1923-24 1924-25
Per cent dead
Average curves for all four strains:
1923-24
1924-25

TEXT-FIG. 2.

It is, of course, true that the months of the years are only roughly comparable and variables occurring within them may bring about such effects as we have described. Future studies along the lines of these observations will doubtless lead to clearer perceptions of such influences. In general, however, the findings of the 2 years are in accord and may be taken to establish the fact that a definite seasonal variation in the susceptibility of different strains of mice to a standard dose of mouse typhoid bacilli does occur.

DISCUSSION.

In our previous communication[3] we expressed the belief that "the seasonal variations in mortality rate recorded for these mice are due to fluctuations in host susceptibility." As a basis for this belief we pointed out that the virulence factor of paratyphoid-enteritidis strains is, as Webster[4,5] has shown, quite constant. In our experiments the dosage factor, an important consideration, was kept uniform. The past year's experience has strengthened our view of the fluctuation of host susceptibility. We have systematically employed for the inoculation a stock strain of M. T. II, kept in agar cultures at +4°C., periodically transferred, and submitted to no animal passage for more than 3 years.

It is mere speculation, in view of the paucity of precise knowledge of the subject, to undertake to explain the seasonal fluctuations which we have ascertained and described, just as at present it would not be profitable to discuss the particular variations of the mortality curves observed in December, 1924, and the 2 following months, from the curves obtained in the previous year.

SUMMARY.

Four unrelated strains of mice were tested over a period of 1 year for their seasonal variation in susceptibility to *per os* feeding with a culture of *Bacillus pestis caviæ* of known virulence. Certain consistent fluctuations, determined to be in general agreement with those already recorded in a previous paper, were found and described.

[5] Webster, L. T., *J. Exp. Med.*, 1923, xxxvii, 781; xxxviii, 33, 45.

THE RELATION OF THE THYROID GLAND TO THE SURFACE TENSION OF THE BLOOD PLASMA.

I. The Effect of Thyroidectomy.

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(*From the Department of Bacteriology and Hygiene of St. Louis University School of Medicine, St. Louis.*)

(Received for publication, October 29, 1925.)

During the course of some experiments on thyroidectomized guinea pigs, we observed that the surface tension of the blood plasma was often considerably higher than that of normal animals. As far as we have been able to learn, no studies have been made of the relation of the thyroid gland to the surface tension of the blood. The present study was therefore undertaken to investigate this question.

Methods of Procedure.

A number of guinea pigs were thyroidectomized under ether anesthesia and the wound closed in layers with linen thread. At various periods after operation, the animals were bled from the carotid artery through a cannula, into graduated centrifuge tubes containing 1 per cent sodium oxalate. One part of oxalate solution to nine parts of blood was used throughout the entire series, although a few preliminary experiments showed that, within reasonable limits, variations in the amount of oxalate did not appreciably alter the surface tension. The blood and oxalate were thoroughly mixed by rapidly revolving the tube between the two palms, and the plasma separated by rapid centrifugalization for about 1 minute. The plasma was always separated from the cells immediately after bleeding, although a few experiments showed that allowing it to remain in contact with the cells for several hours before separation did not appreciably alter the results. Immediately after separation, the clear supernatant plasma was carefully pipetted into watch-glasses and the surface tension determined by means of the du Noüy tensiometer.

The preparation of the glassware constituted one of the most important parts of the work. Every item of glassware used was boiled for 20 or 30 minutes in a freshly prepared solution of potassium bichromate-sulfuric acid cleaning fluid. Following this, it was carefully washed and dried, care being taken to avoid touching with the fingers any part that would subsequently come in contact

with the plasma. Whenever any piece of glassware was in this way contaminated with grease, it was discarded. In most instances, the glassware was cleaned on the same day that it was to be used; occasionally it was cleaned on the preceding day and wrapped in several layers of clean towels until ready for use.

Another feature of considerable importance is the size of the watch-glass, and the amount of plasma used, especially when an interval of time is allowed to elapse between the pouring of the plasma and the determination of its surface tension. Du Noüy[1] has shown that adsorption of the surface-active constituents of serum occurs on the glass as well as at the surface of the fluid. In these experiments, the same size watch-glasses have always been used for the plasma from the operated animal and its corresponding normal control. The watch-glasses which we employed had a diameter of 4.6 cm. and were filled with 2 cc. of plasma.

The tensiometer which we used was not the direct reading type, but was standardized to give the results in dynes according to the method described by du Noüy.[2] The readings were always made by the same person and the greatest care was taken to eliminate any personal factor. The rate at which torsion was applied was kept as nearly constant as possible. It was found necessary to watch the loop closely and to apply the torsion very gradually during the interval just preceding the "break." If this caution is not observed, torsion can be applied in excess of the amount causing the break, and the reading be falsely elevated. A large series of duplicate determinations on the same specimens of distilled water showed that in our hands the method would ordinarily check to within ± 0.1 or 0.3 dyne, and that the maximum variation encountered was ± 0.7 dyne.

Theoretically, the most ideal procedure would have been to study the surface tension before, and at various periods after, thyroidectomy on the same animal. However, when small animals are used (guinea pigs or rabbits), several practical difficulties make this inadvisable. We believe that arterial blood from the same source should always be used. This is important since it is theoretically possible that venous blood, under certain conditions, may have a different surface tension from arterial. Likewise, venous blood, from different, or even the same source, may show fluctuations in surface tension, depending upon the state of activity of certain organs. Because of these considerations, blood was never obtained from the heart, since it is often difficult to be sure of obtaining arterial blood. These difficulties could have been overcome by using larger animals, such as dogs. In these animals, however, the common presence of accessory thyroid tissue scattered throughout the body makes total thyroidectomy almost impossible.

In order to eliminate, or equalize, the effect of all external factors which may

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[1] du Noüy, P. L., *J. Exp. Med.*, 1924, xl, 133.

[2] du Noüy, P. L., *J. Gen. Physiol.*, 1918–19, i, 521.

influence surface tension at liquid-air interfaces (temperature, atmospheric pressure, humidity, etc.) the plasma from each operated animal was always checked with that of a normal animal, done at approximately the same time, and under identical conditions. The temperature at which each pair of determinations was made usually did not vary more than ± 0.5°C. Since the external conditions varied from day to day, comparisons, in the strict sense, are limited to each pair; *i.e.*, the operated and its corresponding normal control.

The studies of du Noüy[3,4] have brought out an important factor which, in the past, has received very little attention; namely, the so called time-drop. He has shown that if serum is allowed to stand, there is a progressive drop of the surface tension, which is in direct relation to the time. Using undiluted serum, du Noüy[5] showed that this drop is very rapid during the first few minutes after pouring. Curves plotting the drop in dynes against the time are nearly vertical during the first 8 or 10 minutes, after which they become nearly horizontal, a slight fall, however, occurring for 2 hours or longer. Since the fall during the first few minutes is so extremely rapid, we believe that more constant results could be obtained by pipetting the plasma into watch-glasses, stirring, and then allowing a definite interval of time (20 minutes) to elapse before determining the surface tension. More recently, du Noüy[6,7] has shown that the injection of an antigenic substance will cause an increase in the value of the time-drop, which becomes evident after about 13 days. In addition, his results also show that the value of the initial reading is frequently, although irregularly, elevated (smallpox vaccination).[7] In our experiments, the operated animals had considerable opportunity to absorb antigenic and other substances which might increase the time-drop (slight infection after operation, autodigestion, and absorption of injured tissue, etc.). The normal controls were not subjected to these possibilities. It is possible that these factors might tend to make the determinations done after a 20 minute interval *lower* in the operated than in the control animals. In view of these considerations, the finding of a *higher* surface tension in our operated animals would be of added significance. After doing a large series in which the surface tension was determined after a 20 minute interval, it was deemed advisable to do a second series in which the initial and 20 minute values were both determined.

In dealing with colloidal solution, only a single determination of surface tension can be made. When the du Noüy tensiometer is used on colloidal solutions, it is not possible to make accurate duplicate determinations on the same specimen. If an interval of time (20 minutes) is allowed for the solution to

[3] du Noüy, P. L., *J. Exp. Med.*, 1922, xxxv, 707.
[4] du Noüy, P. L., *J. Exp. Med.*, 1922, xxxv, 575.
[5] du Noüy, P. L., *J. Exp. Med.*, 1925, xli, 779.
[6] du Noüy, P. L., *J. Exp. Med.*, 1923, xxxviii, 87.
[7] du Noüy, P. L., *J. Exp. Med.*, 1924, xl, 129.

come to equilibrium the determination tears the surface film and disturbs the surface molecular orientation, so that a second reading made immediately afterward will have a higher or lower value—usually higher. If the reading is made immediately after the plasma has been poured, the time-drop is too rapid to permit of a second check determination. Whenever a 20 minute interval was allowed for equilibrium to be established, the solution was covered with a second watch-glass to prevent contamination.

Before considering the experimental data, it is necessary to say a few words about certain difficulties of the operation. The two lateral lobes of the thyroid are usually well encapsulated and sharply defined, so that their total removal is fairly easy. The isthmus, however, presents a different problem. In the guinea pig, the isthmus may be found as a thin, narrow, ribbon-like structure lying over the trachea, or in the form of multiple fine filaments connecting the two lobes. In either case, it is difficult to be certain that it has been totally removed; and if the area is too thoroughly denuded of all suspicious tissue, the parathyroids are apt to be included. Following total thyroidectomy, the animals gain weight rapidly and soon present a puffy, bloated, phlegmatic appearance, which is very characteristic. It was a rather common observation that animals which failed to develop this syndrome did not show elevation of the surface tension of the plasma.

EXPERIMENTAL DATA.

The total series reported here consists of 41 normal controls and 42 thyroidectomized animals. Only male pigs were used and the average weight was from 300 to 420 gm.

We will consider first those experiments in which, before determining the surface tension, a 20 minute time interval was allowed to elapse after the plasma had been pipetted into the watch-glasses. A brief résumé of the results on normal animals will be given before a detailed analysis is undertaken. Out of the 41 normal control plasmas, *regardless of temperature*, only one has a value as high as 58.6 dynes. Two others have values of 56.1 and 56.5 dynes respectively. The remaining 38 have values ranging between 49.4 and 55.4 dynes, the majority being between 52 and 55 dynes. The average value of the 41 controls is 53.2 dynes. On the other hand, the average value for 37 operated animals, tested 18 to 120 days after operation, is 57.3 dynes.

Table I gives the values obtained for 31 normal and 32 operated animals, roughly divided into four groups according to the temperatures at which the determinations were made. Column 3 shows

TABLE I.

Surface Tension of Normal and Operated Animals, Determined after a 20 Minute Period, Divided into Four Groups Depending on the Temperatures at Which the Determinations Were Made.

Temperature.	Normal.		Operated.		Average difference.	Days.
	Surface tension.	Average.	Surface tension.	Average.		
°C.	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	
22–22.5	53.7		58.7			26
	53.4	53.5	59.8	59.2	+5.7	26
25–26.5	51.3		59.5			22
	55.0		58.7			22
	54.9		60.2			22
	55.0		56.5			24
	50.4		53.7			24
			53.7			24
	52.4		58.0			28
	55.4		59.5			28
	53.1		60.4			28
	53.4		61.6			30
	52.6		55.8			30
	54.4	53.4	55.4	57.7	+4.3	30
27–31	54.1		58.6			24
	54.3		58.6			24
	54.3		60.8			24
	52.8		59.4			27
	52.0		53.5			27
	56.5		59.5			27
	52.2		61.1			120
	52.4		53.6			120
	53.9		60.6			121
	53.4		59.5			36
	53.3		57.7			30
	53.4		60.2			31
	55.0	53.6	61.6	58.8	+5.2	31
32–37	50.0		53.0			37
	50.9		52.1			37
	50.6		55.0			31
	50.1		51.4			31
	50.7	50.4	51.2	52.5	+2.1	37

that the average values for the normal plasmas are practically the same at temperatures ranging from 22–31°C.; while at higher temperatures (32–37°C.) the average value is considerably lower. Comparing Columns 3 and 5, it is seen that the average surface tension of the plasma from operated animals is considerably higher than the average value for the corresponding controls, but that the difference is considerably less marked in the last group, which was done at relatively high temperatures (32–37°C.). Since the average values for the normal plasmas are practically identical, in the first three groups, these may be analyzed together. In these twenty-six normal animals, the surface tension of the plasma from twenty-four is below 55.4 dynes, the remaining two are 56.5 and 55.4 dynes respectively. In twenty-seven corresponding plasmas from operated animals, twenty-three have a value of 55.4 dynes or above; the four which are below this figure have values well within normal limits (53.7 (two), 53.5, 53.6). In these four animals the determinations were made 24, 27, and 120 days after thyroidectomy. The failure of these four animals to show the customary elevation of the surface tension may be attributed to at least three possible causes: (1) incomplete thyroidectomy, (2) the presence of accessory thyroid tissue, (3) compensatory activity on the part of other organs. The first of these possibilities appears the most plausible, but the others cannot be definitely eliminated by the data presented here. The determinations on the operated animals were made at periods ranging from 22 to 121 days after thyroidectomy, and it is seen that between these periods the elevation is not in direct relation to the time element; for example, the highest value obtained, 61.6 dynes, occurred in an animal 31 days after operation, while values above 60 dynes were obtained in 22 and 24 day animals.

In the lower half of Chart 1, the first three groups from Table I (22–31°C.) have been plotted with the surface tension in dynes as ordinates. Each normal control is plotted directly below the corresponding operated animal. In general, the curves are fairly well separated and it is seen that neither curve overlaps the average value for the other. In the upper part of the chart, the difference between the surface tension of each operated plasma and its corresponding control is plotted directly above the corresponding points

in the lower curve. This curve shows that the surface tension of the plasma from the operated animals is higher in every instance, and that the differences range from 1 to 9 dynes, with an average of 4.8 dynes.

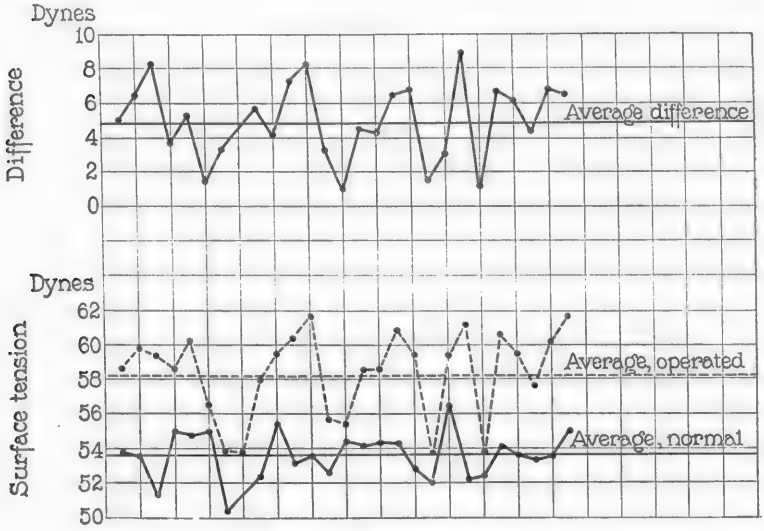

CHART 1.

Lower Half.

..... Operated animals.

——— Normal controls.

All readings were made after the plasma had been pipetted into the watch-glasses and gently stirred. Superimposed points on the two curves represent the values obtained after a 20 minute interval had elapsed, for the operated animals and their corresponding normal controls, done at the same time and under identical conditions. The horizontal line passing through each curve represents the average value.

Upper Half.

Each point on this curve represents the difference between the corresponding normal and operated animals in the lower curves. The horizontal line passing through this curve shows the average difference.

If this change in the surface tension of the plasma is due to removal of the thyroid, then it should be possible to demonstrate that the elevation occurs more or less gradually following thyroidectomy.

TABLE II.

Surface Tension of Normal and Operated Animals, after a 20 Minute Interval, Showing the Gradual Elevation after Operation.

Temperature.	Normal.		Operated.		Average difference.	Days.
	Surface tension.	Average.	Surface tension.	Average.		
°C.	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	
25.5–26	52.8		50.5			6
	54.9		54.3			6
	52.0	53.2	54.9	53.2	0	6
25.5	50.8		54.3			13
	53.5	52.1	53.0	53.6	+1.5	13
24–24.5	54.6		60.2			18
	58.6		53.1			18
	56.1	56.4	54.3	55.8	−0.6	18
30	49.4		54.6			19
	54.3	51.8	58.4	56.5	+4.7	19
26	51.3		59.5			22
	55.0		58.7			22
	54.9	53.7	60.2	59.4	+5.7	22
25	55.0		[56.5			[24
	50.4	52.7	53.7			24
			53.7]	[54.6]	[+1.9]	24]
29	54.1		58.6			24
	54.3		58.6			24
	54.3	54.2	60.8	59.3	+5.1	24
22	53.7		58.7			26
	53.4	53.5	59.8	59.2	+5.7	26
26	52.4		58.0			28
	55.4		59.5			28
	53.1	53.6	60.4	59.3	+5.7	28
25	53.4		61.6			30
	52.6		55.8			30
	54.4	53.4	55.4	57.6	+4.2	30
27	53.4		60.2			31
	55.0	54.2	61.6	60.9	+6.7	31
28	52.2		61.1			120
	52.4		53.6			120
	53.9	52.8	60.6	58.4	+5.6	121

Manipulation of the gland during the operation probably causes an excess of the secretion to enter the circulation. It is known that a single dose of thyroxin exerts a prolonged action extending over a period of 15 days or more. From these considerations, we would anticipate that the elevation of the surface tension would be gradual and would not reach its height until 15 days or more after operation. Table II shows a series of operated animals, with corresponding controls, in which the surface tension was determined at increasing intervals of time after operation. The averages for the separate groups of operated animals are shown in Column 5. The sustained elevation after about the 19th day is broken by one 24 day group, in which the values for two of the operated animals are within normal limits. Column 6 gives the average difference between the corresponding groups of operated and normal animals. It happens that the two highest normal values obtained are in the same group, *i.e.* controls for the 18 day period, so that here the average for the operated animals is less than that of the controls. The values obtained are given without any selection or rearrangement, and it may be considered as a coincidence that the averages for the normal and operated, at the 6 day period, are identical; it is seen, however, that all of the operated animals in this 6 day group are within normal limits. In the 13 day group, both operated animals are likewise within normal limits. The 18 day group shows one out of three which is above the normal, and the 19th day group one out of two. After the 19th day, with the exception of the first 24 day group, the great majority of the operated animals have values which are above the normal figure. In the lower half of Chart 2, the average values for corresponding groups of normal and operated animals are plotted with the surface tension in dynes as ordinates and the days after operation as abscissæ. The gradual rise of the curve for the operated animals is clearly evident. The one 24 day group which is referred to above has not been included in the chart. The distortion of the normal curve at the 18 day period is due, as has been explained above, to the fact that the two highest values which were obtained among the 41 normal animals, occur in this group. The upper half of Chart 2 shows the average difference, in dynes, between the corresponding groups of normal and operated animals.

From these results, it appears that the rise in the surface tension of the plasma after thyroidectomy is gradual, and that it requires about 22 days for the value to rise definitely above the upper limits of normal.

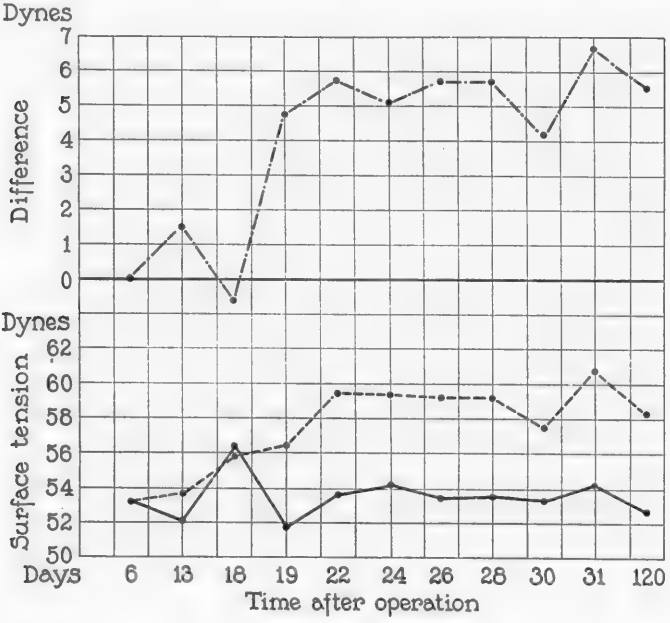

CHART 2.

Lower Half.

..... Operated animals.

——— Normal animals.

The two curves give the average values for groups of operated animals and corresponding controls. The curves show that the elevation of the surface tension after thyroidectomy is gradual, and that it requires from 19 to 22 days to pass beyond the upper range of normal. The determinations were made after a 20 minute interval had been allowed for equilibrium to be established.

Upper Half.

The difference between corresponding groups of normal and operated animals in the lower curves.

After the above experiments had been completed, the question arose as to whether we were dealing with a true elevation of the

surface tension or with a difference in the time-drop. It is possible that the initial value of the surface tension is the same in the plasma from normal and operated animals, but that the time-drop is greater in the normal. In this case, the values obtained after a 20 minute interval would show a difference which would not be present in the initial values. Table III shows the results obtained with eighteen

TABLE III.

Surface Tension of Normal and Operated Animals, Showing the Initial and 20 Minute Values, and the Differences in the Time-Drop.

Temperature.	Normal.			Operated.			Difference in initial.	Difference in 20 min.	Days.
	Initial.	20 min.	Time-drop.	Initial.	20 min.	Time-drop.			
°C.	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	
29	56.9	52.8	4.1	59.5	59.4	0.1	+2.6	+6.6	27
31	54.4	52.0	2.4	58.0	53.5	4.5	+3.6	+1.5	27
31	58.0	56.5	1.5	61.0	59.5	1.5	+3.0	+3.0	27
25	58.0	53.4	4.6	61.7	61.6	0.1	+3.7	+8.2	30
25	58.0	52.6	5.4	58.9	55.8	3.1	+0.9	+3.2	30
25	59.0	54.4	4.6	60.1	55.4	4.7	+1.1	+1.1	30
27	56.0	52.2	3.8	63.9	61.1	2.8	+7.9	+8.9	120
28	59.5	52.4	7.1	61.1	53.6	7.5	+1.6	+1.2	120
28	60.2	53.9	6.3	61.7	60.6	1.1	+1.5	+6.7	121
28	60.6	53.4	7.2	62.4	59.5	2.9	+1.8	+6.1	36
30	58.4	53.3	5.1	60.6	57.7	2.9	+2.2	+4.4	30
27	63.6	53.4	10.2	61.2	60.2	1.0	−2.4	+6.8	31
28	61.7	55.0	6.7	63.4	61.6	1.8	+1.7	+6.6	31
32	52.0	50.0	2.0	58.2	53.0	5.2	+6.2	+3.0	37
33	54.5	50.9	3.6	59.5	52.1	7.4	+5.0	+1.2	37
35	58.3	50.6	7.7	60.4	55.0	5.4	+2.1	+4.4	31
36	55.6	50.1	5.5	59.6	51.4	8.2	+4.0	+1.3	31
37	58.7	50.7	8.0	57.4	51.2	6.2	−1.3	+0.5	37
Average...	57.9	52.6	5.3	60.4	56.7	3.7	+2.5	+4.1	

operated and eighteen normal animals, in which both the initial and 20 minute values were obtained. Here it is seen that the initial surface tension of the plasma from operated animals is higher than that of the corresponding normals in all but two instances. In one of these exceptions, the initial value of the control is so very much above the average value, that it is questionable whether the animal should be considered as normal. If these two exceptions are omitted

the average initial difference between the normal and operated is +3 dynes, while if they are included the average difference is +2.5 dynes. The average initial value for the operated animals is 60.4 dynes and for the controls is 57.9 dynes. Values obtained after a

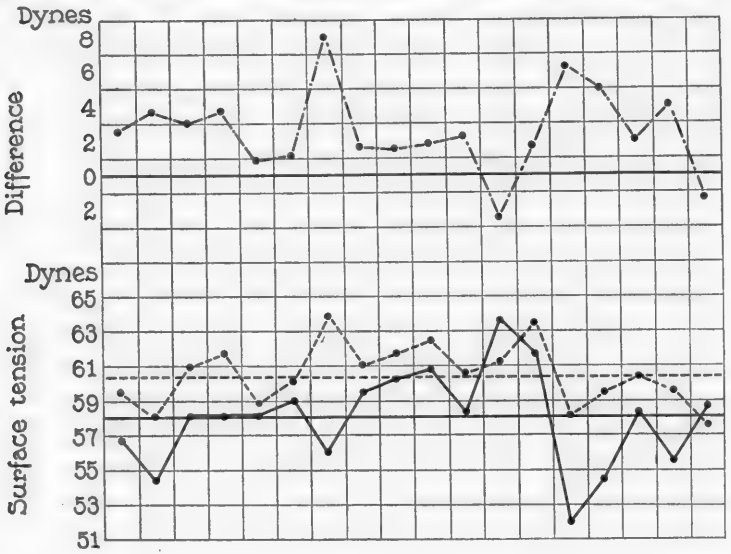

CHART 3.

Lower Half.

..... Opcrated animals.

——— Normal animals.

The readings given here were made *immediately* after the plasma had been pipetted into watch-glasses and gently stirred. Superimposed points on the two curves represent the values obtained for the operated animals and normal controls done at the same time. The horizontal lines passing through each curve represent the average value.

Upper Half.

The difference between each operated animal and its corresponding normal control. The solid horizontal line represents the average difference.

20 minute interval give an average of 56.7 dynes for the operated and 52.6 for the normal.

In general, the time-drop (difference between the initial and 20 minute determinations) is less in the operated than in the normal

animals. The average drop is 3.7 dynes for the operated and 5.3 dynes for the normal. In a few instances, readings were also made after a 2 hour interval and essentially the same differences were observed.

From these results, it appears that the surface tension of plasma from operated animals is higher at both the initial and 20 minute periods. The greater time-drop in normal plasma causes the difference to be more marked in the 20 minute readings. In the lower part of Chart 3, the initial values for the operated and corresponding controls have been plotted with the surface tension in dynes as ordinates. It must be emphasized again that the real comparison is to be made between each normal and the operated animal determined at the same time. In general, the fluctuations of the two curves coincide fairly well. The differences between each corresponding pair have been plotted in the upper half of the chart.

DISCUSSION.

The data presented here seem to establish that there is a true elevation of the surface tension of the blood plasma of guinea pigs following thyroidectomy, but do not explain the mechanism of this change. We suggest the following theory as a working hypothesis. The blood of normal animals contains one or several surface-active substances whose origin is dependent, at least in part, upon the presence of a normal amount of thyroid hormone. In thyroidectomized animals this substance, or group of substances, becomes decreased in amount, so that there is an elevation of the surface tension. The presence of this hypothetical, surface-active constituent in normal blood may be related to the function of the thyroid gland, either directly or indirectly. In the first instance, it may originate, preformed, within the thyroid gland and enter the blood directly, or it may be produced from certain elements of the thyroid secretion after they have left the gland and have entered the general circulation. The second possibility, which we consider more likely, would postulate that this substance, or group of substances, is formed by a few or all of the living cells of the body during the course of their normal metabolism. Following thyroidectomy, the metabolic rate

is greatly diminished, and less of this surface-active element is produced.

By assuming that the plasma from operated animals contains less of certain surface-active elements than normal plasma, we are able to explain the probable mechanism of two rather closely related observations. First, that the time-drop is greater in plasma from normal controls than in plasma from operated animals; and second, that the difference in the surface tension of the plasma from normal and operated animals is more marked after a 20 minute interval has been allowed to elapse than when the determination is made immediately after pouring the plasma (Table III). Surface-active substances bring about a lowering of the surface tension when adsorbed into the surface layer. It is quite evident that the amount adsorbed is at first directly proportional to the time element, thus accounting for the time-drop phenomenon. Within certain limits, however, the amount of the surface-active substance present in the plasma would also play an important rôle. If the quantity was reduced below a certain minimum, it would still be adsorbed into the surface, but the total quantity present in the surface might then be insufficient to form a complete film, so that at equilibrium (after 20 minutes) the surface tension would be higher than when a larger amount is present.

The first step toward a final solution of this problem would be to ascertain whether or not it is possible to *lower* the surface tension by feeding thyroid extract or thyroxin, and whether a soluble substance which exhibits all of the physiological activity of the whole gland extract (thyroxin) is itself surface-active in blood plasma. These phases of the present problem will be dealt with in another paper.

SUMMARY.

1. Following thyroidectomy in guinea pigs, there is a gradual elevation of the surface tension of the blood plasma which reaches its height in from 19 to 22 days after operation. This elevation is probably permanent, since we have found it to persist for as long as 120 days.

2. In our experiments, we found a few thyroidectomized animals in which the surface tension of the plasma was still within normal

limits 24 and 120 days after operation. We suggest that these exceptions are due to three possible factors: (1) incomplete thyroidectomy; (2) presence of accessory thyroid tissue; (3) compensatory activity on the part of other organs.

3. The surface tension of the plasma from operated animals is higher than that from controls in both the initial and 20 minute determinations, but the difference is greater at the 20 minute period.

4. The time-drop (difference between the initial and 20 minute determinations) is somewhat greater in the plasma from normal than in that from operated animals.

5. It is suggested that these changes are due to a decrease in the amount of certain normally occurring surface-active substances, the production of which is directly or indirectly dependent upon the thyroid gland.

THE RELATION OF THE THYROID GLAND TO THE SURFACE TENSION OF THE BLOOD PLASMA.

II. The Effect of the Administration of Thyroid Extract and Thyroxin.

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(Received for publication, October 29, 1925.)

In Part I of this paper,[1] we presented evidence which indicates that there is an elevation of the surface tension of the blood plasma, following total thyroidectomy in guinea pigs. Because of the high mortality following operation, and certain other technical difficulties, it was not practical to study the changes, following operation, in the same animal. It was necessary, therefore, to compare the surface tension of the plasma from thyroidectomized animals with that from normal controls, done at the same time and under identical conditions. Because of the almost perfect antithesis which exists between hyper- and hypothyroidism, we suggested that additional evidence, concerning the influence of thyroid on surface tension, might be obtained by studies of the surface tension before and after the administration of thyroxin and thyroid extract.

Methods and Procedure.

In Part I, we described fully the procedures followed in the preparation of glassware, collection of blood, use of the tensiometer, etc. The same methods have been followed in these investigations and it will not be necessary to repeat the technical details.

In the present studies, the surface tension of the blood plasma from one animal was determined both before and after the oral administration of thyroxin.

[1] Wilhelmj, C. M., and Fleisher, M. S., *J. Exp. Med.*, 1926, xliii, 179.

The blood was always obtained from the carotid artery, was mixed with 1 per cent sodium oxalate solution, and the plasma immediately separated by rapid centrifugalization. Following the first bleeding, the artery was ligated and the wound closed with linen thread. On the following day, the administration of thyroxin was started. After several days of feeding, blood was again obtained from the opposite carotid, and the surface tension determined. In each group, certain animals were left as controls; these were bled on the same days, kept under identical conditions, but received no thyroxin.

In a few preliminary trials, we employed thyroid extract, but in most of the experiments thyroxin was used because it seemed to produce more rapid and constant changes in the surface tension. Tablets containing 0.2 mg. of thyroxin were ground to a fine powder and suspended in water to which a little sugar had been added. The suspension contained 0.2 mg. of thyroxin to the cc. and was fed to the animals with a graduated pipette. The feedings were usually given twice daily, in the morning and again in the evening.

Since several days elapsed between the determinations made before and after feeding, it was sometimes difficult to maintain identical temperatures. In general, however, the temperature differences are small and probably are not sufficient to introduce an appreciable error.

The work of du Noüy[2-4] on the time-drop of serum (difference between the values obtained immediately after pouring and after an arbitrary interval of time) has shown this to be a delicate phenomenon, which is increased by the injection of an antigenic substance. The exact relation which this change bears to the formation of antibodies is not clear. In addition to an increase of the time-drop, du Noüy's results also show that there is frequently an *elevation* of the initial value of the surface tension, following the absorption of an antigen.[5]

The interval of time elapsing between the two determinations (before and after feeding) in our experiments is very short and it is unlikely that within this period the time-drop would be influenced by such factors as slight infection, absorption of injured tissue, etc.; but, nevertheless, this possibility exists. If the time-drop should be increased by any of these factors, the determinations made after a 20 minute interval might show a lowering of the surface tension not due to the effect of thyroxin. In view of these possibilities, we decided

[2] du Noüy, P. L., *J. Exp. Med.*, 1922, xxxv, 575.
[3] du Noüy, P. L., *J. Exp. Med.*, 1923, xxxviii, 87.
[4] du Noüy, P. L., *J. Exp. Med.*, 1925, xli, 779.
[5] du Noüy, P. L., *J. Exp. Med.*, 1924, xl, 129.

to determine the surface tension both immediately after pouring and also after a 20 minute interval, but to consider only the initial value as free from possible secondary influences. We felt further justified

TABLE I.

Difference in the Surface Tension of the Plasma, before and after Feeding with Thyroxin.

Before feeding.				After feeding.				Difference in the same animal before and after feeding.		
Temperature.	Initial.	20 min.	Time-drop.	Temperature.	Initial.	20 min.	Time-drop.	Difference in initial.	Difference in 20 min.	
°C.	*dynes*	*dynes*	*dynes*	°C.	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	
30.5	63.2	60.9	2.3	32.2	54.5	49.8	4.7	−8.7	−11.1	Surface tension determined June 8, 1925. June 9, 10, 11. Received 0.2 mg. of thyroxin in morning and evening. Surface tension determined June 12.
30	64.4	60.1	4.3	31.5	60.4	51.0	9.4	−4.0	−9.1	
29.5	63.7	60.2	3.5	30	59.4	51.4	8.0	−4.3	−8.8	
31	61.1	53.5	7.6	31.7	58.7	51.7	7.0	−2.4	−1.8	
32	61.1	53.4	7.7	32	52.4	49.8	2.6	−8.7	−3.6	
31	61.6	58.2	3.4	31	61.2	54.2	7.0	−0.4	−0.4	Control. No thyroxin. Was sick during the entire interval between determinations.
32	61.8	53.7	8.1	31	57.4	52.0	5.4	−4.4	−1.7	Surface tension determined June 15, 1925. June 16, 17, 18. Received 0.2 mg. of thyroxin in morning and evening. June 19. No feeding. June 20. Surface tension determined.
35.5	60.8	52.8	8.0	32.5	57.5	50.5	7.0	−3.3	−2.3	
35	61.0	53.4	7.6	33	58.6	51.2	7.4	−2.4	−2.2	
33	61.0	52.7	8.3	32	60.2	53.4	6.8	−0.8	+0.7	
32	61.0	52.7	8.3	32	61.7	52.7	9.0	+0.7	0	
32	61.2	52.6	8.6	31.2	61.1	52.8	8.3	−0.1	+0.2	Control. No thyroxin.

The first three experimental animals in this table all show initial and 20 minute values which are considerably higher than of those any of the other animals used. They had been used some time before for the standardization of diphtheria toxin, but had survived. We have noted this elevation in similarly treated animals on several other occasions. All other animals used in the present experiments are normal.

in this decision by the findings in one control animal which had received no thyroxin, but which was sick during the entire interval between the two determinations.

In this animal the initial value of the surface tension was practically the same in the two determinations (0.4 dyne lower in the second), while the 20 minute value showed a decrease of 4 dynes (see Table I).

In comparing the values for the surface tension, before feeding thyroxin, with the values given in Part I for the normal controls, one is impressed by the fact that the former tend to be somewhat higher. The data reported in Part I of this paper were obtained during midwinter, while the present experiments were conducted during the months of June and July. Our work on the surface tension of guinea pig plasma extended over the period from December to August, and we observed that there was apparently an increase in the normal value as the weather became warmer. The cause for this shift in the normal value is not clear. If temperature was the only factor involved the reverse effect would be expected. No definite explanation can be given, but it is necessary to call attention to age difference and seasonal variations as possible factors.

EXPERIMENTAL DATA.

Only male pigs were used and the average weight varied from 300 to 420 gm.

Since most of the experiments were made with thyroxin, these will be discussed first.

The total amount of thyroxin administered varied from 1 to 1.2 mg.; and the surface tension was determined on the 4th and 5th days after the feeding was started. Since it requires from 3 to 4 days for a single dose of thyroxin to become active, and since the quantity absorbed from the intestine is variable, it is impossible to say just how much has been effective in these experiments. A few of the animals showed practically no symptoms, while the remainder were, in general, only slightly affected. In some, there appeared to be a slight loss of weight and general weakness. The most common symptom was tachycardia with a pounding cardiac impulse, which was not observed in the unfed controls. One animal, in particular, showed symptoms of intoxication with marked loss of weight and extreme weakness. In this animal we observed the greatest drop in the surface tension; *i.e.*, 13.9 dynes. With one exception (Table I), the controls remained healthy during the interval between the two determinations.

In the tables, the initial and 20 minute values and also the time-drop are given; but in the analysis we will limit ourselves to a con-

sideration of the initial values. The results are given in Tables I to III. The mean values for the entire series are shown at the end of Table III. Out of twenty-seven animals which were fed thyroxin, twenty showed a fall in the initial value of the surface tension, after the feeding, which was greater than 1 dyne. The decrease in the surface tension of the plasma of these twenty animals varied from 1.9 to 13.9 dynes, with a mean value of 5 dynes. Six of the remaining

TABLE II.

Difference in the Surface Tension of the Plasma, before and after Feeding with Thyroxin.

Before feeding.				After feeding.				Difference in same animal before and after feeding.		
Temperature.	Initial.	20 min.	Time-drop.	Temperature.	Initial.	20 min.	Time-drop.	Difference in initial.	Difference in 20 min.	
°C.	*dynes*	*dynes*	*dynes*	°C.	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	
26.5	61.7	57.3	4.4	26.5	59.8	53.4	6.4	−1.9	−3.9	Surface tension determined June
27.6	59.5	52.2	7.3	27	58.8	51.3	7.5	−0.7	−0.9	25, 1925. June 26, 27. Re-
27.7	59.5	54.7	4.8	28	60.2	53.2	7.0	+0.7	−1.5	ceived 0.2 mg. thyroxin in
28	63.1	58.2	4.9	28	60.2	53.2	7.0	−2.9	−5.0	morning and evening. June
28.5	62.4	58.6	3.8	28.5	52.1	47.2	4.9	−10.3	−11.4	28. No feeding. June 29.
30	61.7	52.7	9.0	29	56.5	49.8	6.7	−5.2	−2.9	Received 0.2 mg. thyroxin in
30	61.5	58.3	3.2	29	58.7	52.8	5.9	−2.8	−5.5	morning. June 30. Surface
31	61.0	53.1	7.9	31	58.6	50.9	7.7	−2.4	−2.2	tension determined.
27	60.2	56.7	3.5	27	60.7	55.4	5.3	+0.5	−1.3	Controls. Received no thy-
30.5	61.8	52.0	9.8	30	61.6	53.0	8.6	−0.2	+1.0	roxin.
32	61.9	53.0	8.9	29	62.5	57.2	5.3	+0.6	+4.2	

seven animals showed differences in the surface tension before and after feeding varying from an increase of 0.7 of a dyne to a decrease of 0.8 of a dyne, while one showed an elevation of 1.1 dynes. Among seven control animals, which were bled on the same days and kept under identical conditions, six varied from 0.5 dyne increase to 0.6 dyne decrease on the two determinations, while one showed a fall of 1 dyne.

In the lower half of Chart 1, the results from the twenty animals

which showed a drop from the initial value greater than 1 dyne have been plotted with the surface tension in dynes as ordinates. The upper, unbroken curve represents the values obtained *before* feeding

TABLE III.

Difference in the Surface Tension of the Plasma, before and after Feeding with Thyroxin.

Before feeding.				After feeding.				Difference in same animal before and after feeding.		
Temperature.	Initial.	20 min.	Time-drop.	Temperature.	Initial.	20 min.	Time-drop.	Difference in initial.	Difference in 20 min.	
°C.	*dynes*	*dynes*	*dynes*	°C.	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	
34	58.2	53.2	5.0	34.2	52.2	49.5	2.7	−6.0	−3.7	Surface tension determined July 15, 1925. July 16, 17, 18. Received 0.2 mg. thyroxin in morning and evening. July 19. No thyroxin. July 20. Surface tension determined.
33.5	60.8	53.0	7.8	33.5	58.2	50.5	7.7	−2.6	−2.5	
32.7	60.6	52.7	7.9	32.5	51.2	47.6	3.6	−9.4	−5.1	
32.7	60.8	53.6	7.2	32.6	57.9	51.0	6.9	−2.9	−2.6	
31.3	61.4	52.8	8.6	31.7	59.0	51.9	7.1	−2.4	−0.9	
31.3	60.8	52.7	8.1	31.3	60.1	52.5	7.6	−0.7	−0.2	
31	60.7	52.8	7.9	31.3	46.8	43.1	3.7	−13.9	−9.7	
33.7	57.9	53.5	4.4	33.3	57.1	50.7	6.4	−0.8	−2.8	
30.3	60.5	52.8	7.7	30.8	61.6	51.2	10.4	+1.1	−1.6	
34	61.6	52.1	9.5	34	60.6	51.8	8.8	−1.0	−0.3	Controls. No thyroxin.
34	61.0	52.4	8.6	34	60.5	52.1	8.4	−0.5	−0.3	
	61.2	54.6	6.5		57.4	50.8	6.6	−3.7	−3.7	Average for *all* animals which had received thyroxin.
	61.3	53.8	7.5		61.1	53.8	7.4	−0.15	−0.07	Average for unfed controls.
	61.5	55.1	6.3		56.5	50.4	6.1	−5.0	−4.8	Average for the 20 fed animals which showed a drop greater than 1 dyne in the initial value after feeding.

and the lower, broken curve those *after* feeding. Superimposed points represent the two determinations from the same animal. In the upper half of Chart 1, the seven control animals have been plotted on the same scale. The solid curve connects the values for the first determination and the broken curve those obtained on the second deter-

mination. Here, also, superimposed points represent the values obtained on the same animal.

The seven animals which showed practically no change in the surface tension after feeding with thyroxin present an interesting problem, and their behavior cannot be explained on the basis of the

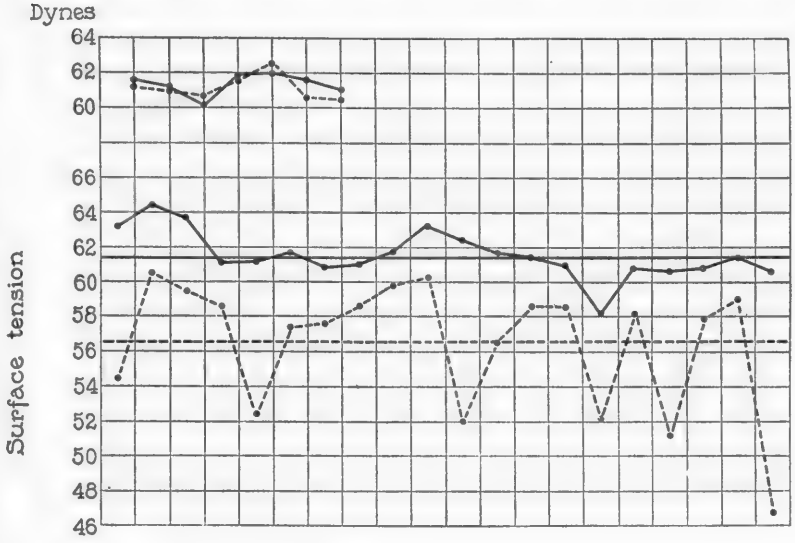

CHART 1.

Lower Half.

——— Before feeding thyroxin.

– – – – – After feeding thyroxin.

Superimposed points on the two curves represent the values obtained on the same animal before and after feeding thyroxin. The horizontal lines passing through the two curves represent the average values. The determinations were done *immediately* after the plasma had been pipetted into watch-glasses and gently stirred.

Upper Half.

——— First determination.

– – – – – Second determination.

The curves show the values obtained on seven control animals which were bled on the same days and kept under identical conditions, but received no thyroxin. Superimposed points on the two curves represent the two values obtained on the same animal. The determinations were made immediately after the plasma had been pipetted into watch-glasses and gently stirred.

present experiments. We suggest as possibilities, however, that the thyroxin was not absorbed from the intestine in sufficient quantity to bring about the change, or that a balanced compensation prevented it.

In only one instance (Table III) did we observe what can be considered as a significant elevation after feeding (1.1 dynes). This may, of course, represent a purely technical error, but in the present state of our knowledge we must admit of the possibility of overcompensation in the early stages of thyroid intoxication.

TABLE IV.

Surface Tension after Feeding Thyroid Extract, Showing the Rise to Normal in One Animal after Feeding Had Been Discontinued.

	Initial	20 min.	Remarks.
	dynes	*dynes*	
1	48.3	47.4	Fed pulverized thyroid extract tablets, Burroughs Wellcome and Co., 2½gr. for 10 days as follows:
2	47.8	47.6	First 6 days—32 mg. daily. 7th day—no feeding. 8th " —64 mg. 9th " —64 " 10th " —96 " Showed marked emaciation, weakness, diarrhea, and tachycardia.
2	58.6	52.3	Animal 2 of the above group survived and was given no thyroid extract for 11 days. During this period, it gained weight and became much stronger. Surface tension determined on the 11th day, after feeding had been discontinued.

In a few preliminary experiments we fed thyroid extract instead of thyroxin, but determined the surface tension, however, only *after* the period of feeding. The values obtained in these cases were either at or below the lower limit of normal. In Table IV, two of these experiments are shown in which the immediate values of the surface tension, after feeding, were 48.3 and 47.8 dynes, respectively. Both of these animals showed extreme emaciation, weakness, diarrhea, and tachycardia. Animal 2 survived and thyroid feeding was dis-

continued for a period of 11 days. During this interval, the animal gained weight and showed considerable amelioration of all previous symptoms. At the end of 11 days, the surface tension was again determined, and the initial value found to be within normal limits, 58.6 dynes, an elevation of 10.8 dynes from what had been observed during feeding.

Could the presence of thyroxin, *per se*, in the blood account for the observed lowering of the surface tension? In order to determine this, we tried adding small amounts of a concentrated solution of thyroxin crystals to fresh guinea pig plasma. The results were altogether irregular, and showed either no change, a slight elevation, or a slight lowering of the surface tension. Apparently, thyroxin is not strongly surface-active *in vitro*, and is, therefore, probably not directly responsible for the changes observed *in vivo*.

DISCUSSION.

At the present time too little is known regarding the rôle of complicated physicochemical factors in governing metabolism or cellular activity in the living body, to permit us to speculate on the part played by the surface tension of the blood plasma. This physical constant, however, is undoubtedly a factor of some importance in all activities involving diffusion and osmosis, and probably plays no inconsiderable rôle in many enzymatic activities. We would, therefore, by analogy expect that the living organism would possess the power to minimize or prevent changes in the surface tension of the blood. By comparison with other better known regulatory activities, it would be expected that these compensatory or protective adjustments might be of two kinds; one type, purely physicochemical in nature, more or less independent of vital activity, and the other directly associated with functions of living cells.

Du Noüy[6] has clearly demonstrated at least one phase of the purely physicochemical mechanism which protects the organism against changes in the surface tension of the blood. He has shown that when a strong surface-active substance, such as sodium oleate, is added to blood serum *in vitro*, there is a drop in the surface tension which is immediately followed by a rise, so that the normal value

[6] du Noüy, P. L., *J. Exp. Med.*, 1922, xxxvi, 115.

is regained within a very few minutes. This phenomenon is due to the fact that the sodium oleate molecules are adsorbed by the colloidal particles and withdrawn from the surface. He has also shown that this buffing action is most marked against concentrated sodium oleate. When a dilute sodium oleate solution was used, he found that the recovery was slow and sometimes incomplete. He further observed that the surface tension *after* recovery often reached a *higher* value than *before* the addition of sodium oleate. He explained this by assuming that the sodium oleate molecules formed "a thick coating around the molecules or group molecules of serum, which tends to force them apart a little more than they were before." We have referred to a process of this kind by the term overcompensation.

In addition to this type of purely physicochemical compensation, the cells of the living organism may possess the power, at least in some instances, either to excrete from the body, or to change chemically a surface-active substance into one which is inactive. Variations in these stabilizing or protective mechanisms may partially explain why, in our experiments, some animals would show a marked decrease in the surface tension while others remained practically unchanged.

As a working hypothesis, we have suggested that the changes in the surface tension of blood plasma, caused by thyroidectomy or the feeding of thyroid extract and thyroxin, are due to changes in the quantity of certain normally occurring surface-active substances. Since it is possible to cause a definite lowering of the surface tension by feeding thyroxin, the probable origin of this surface-active constituent is reduced to two sources; either it is produced from thyroxin which is in some manner changed after it has entered the blood stream and come into contact with living cells, or, what is more probable, the surface-active substance is produced during the course of normal metabolism by certain cells of the body. It is quite likely that many of the final or intermediate products of cellular activity are surface-active, and an increase in the rate of cellular metabolism such as would follow the administration of thyroxin would lead to an increased production of these elements.

The data reported in this paper have been gathered with a view toward answering only one question: Is it possible to cause a lowering of the surface tension of the blood plasma by feeding thyroid extract or thyroxin? In attempting to answer this question, we have avoided many closely associated problems. One of the most important of these is the investigation of the time factor and its relation to the results reported here. For example, the question arises: Is the depression

of the surface tension a permanent condition which will persist as long as thyroid feeding is maintained, or is it transitory, occurring only during the first few days, when possibly the body has not yet adjusted itself to the abrupt increase in metabolism? In the latter instance, we might expect to find that the initial depression would be followed by a return to normal as certain compensatory adaptations were perfected.

SUMMARY.

1. Determinations of the surface tension of blood plasma from guinea pigs, before and after feeding thyroxin, showed in most cases a definite decrease. Out of twenty-seven experimental animals, twenty showed a depression in the surface tension which ranged from 1.9 to 13.9 dynes, with an average of 5 dynes. Six animals showed variations hardly exceeding the limits of experimental error, while one showed an elevation of 1.1 dynes.

2. Six normal control animals, bled on the same days and kept under identical conditions, showed differences in the two determinations which ranged from a decrease of 0.5 to an increase of 0.6 of a dyne. One other control showed a drop of 1 dyne.

3. Two animals which had been fed large quantities of thyroid extract over a period of 10 days, gave values considerably below normal. One of these animals survived, and after 11 days during which no thyroid extract was given, the surface tension was found again to be within normal limits.

4. The hypothesis is advanced that these changes are due to an increase in the amount of certain normally occurring surface-active constituents, which are produced as a result of increased cellular metabolism.

THE RELATION OF BACILLUS ABORTUS FROM BOVINE SOURCES TO MALTA FEVER.

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The existence of races indistinguishable fundamentally and differing only in minor physiological characters has presented a major problem to comparative pathologists since the methods of Koch have come into use. The paratyphoid, the tubercle bacillus, and the streptococci are well known examples. To this group must now be added the *melitensis-abortus* species. The races of tubercle bacilli have definite hosts and this fact is of great value in preventing confusion. The paratyphoid races, undoubtedly originating or developing in different host species, have not yet been definitely referred to their respective hosts. The *abortus* species also has definite hosts to which the races may be referred, the cow, the goat, and the pig. Perhaps races may be found in the future allied with other animals.

The practical significance of these host relationships bears upon the occasional invasion of other hosts. Statements based on recent comparative studies of *B. abortus* and *B. melitensis* have been made to the effect that these two organisms are identical and that a clinical complex similar to Malta or undulant fever may be produced in man by *B. abortus*. On the other hand, the well defined geographical limitations of Malta fever and its relation to goat's milk, the high degree of infectiousness of *B. melitensis* towards laboratory workers handling this organism, and the wide diffusion of infectious abortion in cattle throughout Western Europe and the United States, all militate against this identification. They make it desirable to analyze more minutely any differences that may exist between these races and to trace more definitely the source of infection in the few sporadic cases of Malta fever in man occurring in regions where goat's milk and its products do not openly appear in the markets.

The close relationship between *B. abortus* and *B. melitensis* in morphological, biological, and serological characters was first pointed out by Alice C. Evans in 1918 (1). Although *B. abortus* was discovered by Bang in 1897 and *B. melitensis* by Bruce in 1891, and although the fundamental relation between these organisms is such that it could hardly have been overlooked had the two organisms come under the observation of the same person, the bringing together had to wait many years because of the hitherto separate flow of research in human and animal diseases. The essential identity of the two organisms pointed out by Evans has been affirmed by all those who have subsequently compared them. The problem to be solved concerns the minor differences which make one pathogenic for man, the other not, except perhaps under unusual conditions still to be defined.

Besides the use of immunological reactions in a comparative study of races or varieties the use of animals may be of decisive importance, as was shown by the writer in distinguishing between mammalian races of the tubercle bacillus. The problem is to choose an animal in which differences, if such exist, are most highly magnified and brought out regularly and without much variation.

Fleischner, Vecki, Shaw, and Meyer (2) carried out a number of experiments on several species of monkeys with *B. abortus* and *B. melitensis*. They used intravenous inoculation and feeding in milk and on solid foods. They conclude that although virulent strains of *B. abortus* may be pathogenic for monkeys, *B. melitensis* is far more invasive.

Burnet and Lagoanère (3) inoculated rats and mice with both organisms but they were unable to find any differences. Durham (4) attempted to produce a fatal disease in guinea pigs by injecting a large dose into the cerebrum and raise its virulence by passages.

Eyre (5) describes an acute and a chronic disease in guinea pigs resulting from intracerebral inoculations. In the chronic disease the animal is distinctly ill for 3 to 6 days but gradually recovers. After an interval of weeks or months during which the animal appears in perfect health, death suddenly takes place. No characteristic lesions are described by Eyre. The urine was usually infected.

Nicolle and Conseil (6) describe a spontaneous infection of guinea pigs with *B. melitensis* in a goat stable in Tunis. Somewhat later the same authors (7) inoculated two guinea pigs and fed two others with a culture of *B. melitensis* recently isolated from a goat. One of each pair was killed on the 42nd day. *B. melitensis* was isolated from spleen, blood, bile, but not from liver. The urine

was not examined. The other pair was killed on the 134th day. There was slight hypertrophy of the spleen. Cultures were obtained from spleen and urine.

In 1911, the writer and Fabyan (8, 9) first described a slow, progressive disease in guinea pigs following the injection of *B. abortus* and having quite characteristic lesions. Since that date, guinea pigs have been used quite regularly for the isolation of *B. abortus* from uterine fluids and milk containing a variety of bacteria.

Khaled (10) states that ¾ of a slope of *B. melitensis* killed a guinea pig of 240 gm. in 18 hours. To kill a guinea pig of 240 gm. in approximately the same time, 4 ½ slopes of *B. abortus* were required, thus showing that *B. melitensis* is about 6 times as virulent as *B. abortus*. A history of the cultures is not given.

K. F. Meyer, Shaw, and Fleischner (11) studied comparatively the lesions produced by *B. abortus* and *B. melitensis* in guinea pigs. The method used was to inject the cultures directly into the testicles, the dose being 1/5 to 1/20 of an agar slant, or from 1 to 2 billion bacteria. The results with *B. melitensis* were that "four cultures . . . . infected guinea pigs quite regularly, while eighteen other strains either proved non-pathogenic or occasionally produced lesions in the spleen, lymph nodes, etc." The disease produced by *B. abortus* was either acute or chronic and varied from strain to strain. It involved the spleen, lymph nodes, testicles, and joints. They conclude that "*B. abortus* is as a rule slightly more invasive and virulent than *B. melitensis*."

Jaffé (12) in the same year published results of similar import. He injected strains of *B. abortus* and *B. melitensis* into the testicles, ovaries, the peritoneal cavity, and the subcutis. His cultures, four of each race, came from Kral's collection. The dose used was 1/10 of an agar culture. Jaffé finds the lesions caused by both races much the same, although those due to *B. abortus* were more pronounced.

Et. Burnet (13) in discussing the lesions in guinea pigs due to inoculation with *B. melitensis* mentions hypertrophy of spleen and invasion of the marrow of the long bones. 5 per cent of the inoculated animals developed paraplegia. The histological changes are not discussed. Jaffé states that endothelial foci are produced by *B. abortus* and *B. melitensis* and that necrosis does not occur either in testicles or spleen. K. F. Meyer, Shaw, and Fleischner (11) describe the lesions due to *B. melitensis* as "nests of epithelioidal and scattered giant cells . . . . common to all focal lesions in the lymph nodes, spleen, and liver. . . . . The pathologic process is indistinguishable from that seen in guinea pigs affected with *B. abortus*."

Bearing upon the relation between *B. abortus* and *B. melitensis* the experiments of Nicolle, Burnet, and Conseil (14) are of interest. They injected subcutaneously 800 to 900 million living bacilli from 24 hour cultures of *B. abortus* into five human beings. No fever or other troublesome symptoms followed. They suggest the use of *B. abortus* in treating Malta fever.

Polettini (15) has recently described an anaphylactic test which is to distinguish between the bovine and the caprine race. The dried and powdered growth from agar cultures is suspended in a 1 per cent solution of anhydrous sodium carbonate

in distilled water. Rabbits received into an ear vein 1 to 5 cg. of the suspended powder of either strain. There was no change during ½ hour's observation of the blood pressure, respiration, or coagulability of the blood. They then received seven injections of the same dose subcutaneously 1 week apart. 12 days after the last injection they received 2 cg. into an ear vein. When *B. melitensis* followed treatment with *B. abortus* there was noticed within 1 ½ minutes after intravenous injection a fall in blood pressure from 180 to 130 mm., rise in respiration to 100 per minute, and coagulation of the blood in 60 minutes. These changes did not occur when *B. abortus* followed treatment with *B. abortus*.

Assuming that the observers failed to present any salient distinguishing characters between *B. abortus* and *B. melitensis*, the writer considered the subject still open. Two conditions probably interfered with clear-cut results. One is the use of old cultures, and the other the injection of too large doses. The inoculation disease in guinea pigs is a slow, chronic process and has many characters like tuberculosis. All who have worked with tubercle bacilli know that the acute disease following large doses is very different from the chronic type due to minute doses. It is the latter type which is of value in the recognition of races and varieties.

The desirability of determining more definitely the relation between *B. abortus* isolated from cattle and the races isolated from human subjects manifesting certain well defined symptoms which are here called Malta fever for convenience is emphasized by the great economic importance of *B. abortus* in the dairy industries and the serious situation which might be created if it were assumed that *B. abortus* produces a diseased condition in man. Fortunately a human culture recently isolated in New Haven and kindly offered by Dr. Francis G. Blake was available for a comparative study. This strain is designated *B. melitensis* II. Two separate cultures from the same patient were tested as II_a and II_b and found identical. At the same time an older culture isolated from a case in Baltimore and described by Keefer (16) was drawn into the comparative study through the kindness of Dr. Harold L. Amoss. This strain is designated *B. melitensis* III. It was isolated in 1922.

Although hundreds of strains of *B. abortus* have been isolated and passed through guinea pigs in this laboratory since 1917, it was deemed best to associate with these two strains of *B. melitensis* two

of *B. abortus*, both isolated through guinea pigs in September, 1924. One (1114) had a high CO_2 requirement, *i.e.* it multiplied very slowly in sealed tubes and not at all in open tubes, while the other (1119) was far less exacting and multiplied in unsealed tubes but only in low dilutions.

The chief characteristic features of the *B. abortus* disease in guinea pigs having been described some years ago (8, 9) it is in order to summarize them briefly, in view of a further prolonged study of them since 1917.

In guinea pigs receiving minute doses of recently isolated strains or material from bovine uterus or fetus the course of the disease and the resulting lesions are quite uniform in character. Usually there is a slight retardation in the normal weight curve during the 1st and 2nd weeks. Thereafter the weight advances as in the normal animal. There is no local swelling, abscess, or ulcer and only a slight enlargement of the regional lymph nodes.

Information obtained from guinea pigs killed at different periods following inoculation brings out a certain progression in the lesions observed. The spleen begins to enlarge in the 2nd week and continues up to the 8th or 10th week. The enlargement is mainly a congestion due to the distension of the sinuses and following the appearance of endothelial foci. The organ becomes very rich in blood. Minute grayish foci appear, often in large numbers, which rarely exceed 1 mm. in diameter and remain grayish and firm. These are often concealed by the congestion. The linear spleen dimensions may be three times the normal. When the congestion remains slight, the surface of the organ is no longer smooth but slightly nodular.

After the 4th week minute, yellowish depressions dotting the liver surface appear. They may be very few or fairly numerous. The subcutaneous lymph nodes become swollen but remain inconspicuously buried in the local fat deposits and do not show any distinct macroscopic changes.

After the end of the 2nd month other localizations may appear. The kidneys become pale, or even white, and enlarged, due to interstitial foci coalescing and leading to a slow destruction of the cortical tissue. The carpus or tarsus may be swollen, ribs enlarged and rarefied. The ciliary region of the eyes may become infiltrated and paralyses due to localizations in the spine and membranes of the cord may appear quite suddenly. These special localizations were far more frequent in the material from Massachusetts studied in 1910 and 1911 than in the more recent investigations from 1917 on. There is here presented the possibility of a gradual modification and attenuation of the virus in the bovine species during this period, or else the regional occurrence of types slightly differing from one another.

Lesions of the testicles are rarely absent after the 2nd month and they may

appear soon after the 1st. The epididymis is the chief site. The interstitial tissue becomes infiltrated and it compresses and obliterates the tubules. The entire focus becomes permeated with polymorphs and converted into a cheesy mass. The dominant lesion is an infiltration of spleen and lymph nodes with cells of endothelial type, forming roundish tubercles or more diffuse meshworks.

B. abortus is present in the spleen in largest numbers during the 3rd and 4th weeks (17). After the 8th week, the spleen may be very large but the bacilli scarce. Cultures may fail at this stage if old strains have been injected. They usually fail unless bits of spleen of pea-size, or even larger, are introduced into the culture tubes.

As stated above, the disease in guinea pigs resembles in many respects tuberculosis in the same species, excepting that the tubercular guinea pig dies sooner or later whereas the *abortus* guinea pig recovers, or more accurately does not become visibly ill at all except for occasional localizations in the bones, the eyes, the cord, and the kidneys. Death has been observed as a result of paralysis and of rupture of the enlarged and congested spleen.

EXPERIMENTAL DATA.

Two recently isolated strains of *B. abortus* were inoculated into guinea pigs in the same doses used in studying the effects of the two strains of *B. melitensis*.

To prepare the culture for injection, a fresh agar slant was inoculated, sealed, and allowed to develop into a uniform grayish film which usually reached its height in 48 to 72 hours. The sealing tends to insure uniformity of growth in the cultures to be compared. If tubes are left open, strains not requiring CO_2 grow more richly than recently isolated *B. abortus* strains. The seal restrains the former and assists the latter. The resulting films are therefore much the same quantitatively. The tubes used measured about ⅝ inch and contained 6 cc. of nutrient agar. The sealing is done with sealing wax and requires care since even minute breaks modify the growth.

The film is washed down with the condensation water. This was about 1 cm. deep and ⅓ cc. in volume. The condensation water becomes heavily turbid and contains the entire mass of bacilli. This was diluted twice in 10 cc. normal saline with the aid of a platinum loop holding about 0.004 cc. The second dilution contained in 1 cc., 0.00000016 cc., or $\frac{1}{6,250,000}$ cc. of the condensation water. Multiplying this figure by 3, we obtain the dilution of the entire culture suspension since the condensation water measures only ⅓ cc.

The guinea pigs received either ½ cc. or 1 cc. into the subcutis or peritoneal cavity. Twenty-one guinea pigs, in all, were inoculated

with the minute doses of *B. melitensis* II and III and killed at intervals beginning with the end of the 3rd week and ending with the 11th week. All animals survived except one which died following a rupture of the diaphragm due to some accident. An equal number were inoculated with the same amounts of *B. abortus*, Strains 1114 and 1119. Besides these a number of guinea pigs inoculated with fresh material from the bovine disease received during the investigation were included in the study.

The course of the disease due to the two strains of *B. melitensis* was much the same. *B. melitensis* II was, however, more virulent in that the lesions were more extensive. This may perhaps be explained by the fact that *B. melitensis* III had been under cultivation about 2 years when the present inoculations were made, whereas *B. melitensis* II was only 4 months old.

Comparing the changes in weight of the two groups—*B. melitensis* on the one hand, *B. abortus* on the other—the average gain of the former in a period of 5 weeks was about 100 gm. per animal, that of the *B. abortus* group 200 gm. After 9 weeks the average gain of the eight *B. abortus* animals was 300 gm., that of the seven surviving *B. melitensis* animals 197 gm. The average gain of the *B. melitensis* group II was 103 gm., that of the *B. melitensis* group III 242 gm.

The mode of inoculation, whether into subcutis or peritoneal cavity, determined to a certain degree the extent and age of the lesions in the lymph nodes. The subcutaneous inoculation usually produced suppurating kneefold nodes up to 1 cm. in diameter. After 9 or 10 weeks these were mere sacs filled with a cheesy mass which emerged from the incision under pressure as a tape-like mass. The involvement of the other superficial lymph nodes varied from animal to animal. The most frequently attacked nodes were the cervical lymph nodes and the thymus, next the axillary. In these glands there was a variable number of roundish, yellowish foci from 1 to 3 mm. in diameter. In one animal both thymus glands were the seat of foci 1 cm. in diameter. In all cases the foci were centrally softened or cheesy after 4 weeks. The bronchial node at the apex of the thorax became swollen later, but necrotic foci were not observed.

The spleen during the early weeks became enlarged and congested

and resembled closely the *B. abortus* spleen. In some animals it was permeated with large numbers of minute grayish foci. Later a smaller number enlarged and took on the appearance and consistency of the lymph node foci described above.

The lesions of the testicles were scarcely distinguishable from those of the *B. abortus* disease, because in both the localization is the same and softening of the foci occurs after a time. The lesions due to *B. melitensis* were in some cases associated with extensive edema, probably because of involvement of the pelvic lymph nodes. The contents of the seminal vesicles became clouded and opaque due to suppurative infiltration, a condition seen up to the present in only one *B. abortus* animal. In both groups, minute, depressed, grayish or yellowish point-like foci were present in the liver.

In the guinea pigs kept for 10 or 11 weeks, one out of eight *B. abortus* animals developed a swollen carpal joint. Of the eight *B. melitensis* guinea pigs, one had a swollen tarsal joint and one paralysis of the hind limbs.

The histological character of the lesions is best seen in the lymph nodes. The foci of endothelial elements cause a rarefaction of the greater portion of the gland tissue on account of the poverty in chromatin of the nuclei and the feebleness or absence of any cytoplasmic stain. On the periphery of the nodes there is still a narrow zone of normal lymphocyte elements. The changes which the neoplastic areas undergo in *B. melitensis* infection after the 3rd week are limited to foci not smaller than 0.6 mm. in diameter. These become centrally occupied by polymorphs which form a distinct nucleus. The larger foci or those areas formed by the union of several foci may undergo central necrosis either before or after invasion by polymorphs. In the latter case the necrotic central mass is densely filled with nuclear debris of polymorphs. In the former case the necrotic center contains but little nuclear debris and it is surrounded by a demarcating wall of partly necrotic polymorphs.

Liver foci in the guinea pigs inoculated with the bovine cultures remain very small. They are not more than 1 mm. in diameter, usually slightly depressed and of a yellowish color. They are not very characteristic histologically and are evidently metastases from the spleen leading to necrosis of a few liver cells and subsequent

infiltration with lymphocytes and a few endothelial elements. In the guinea pigs inoculated with *B. melitensis* liver foci appear and later on tend to enlarge and become centrally softened.

B. abortus in Swine.—In view of the results obtained it seemed necessary to look elsewhere for the source of the human infections. The existence of a race of *B. abortus* causing abortion in swine was first pointed out by Good and Smith in 1916 (18). In 1914 Traum[1] had isolated *B. abortus* from the organs of an aborted fetus. Since then the existence of infectious abortion in swine has been observed by others in the Middle West. The bacillus isolated resembled the bovine type and agreed with it serologically. It grew, however, in unsealed cotton-plugged tubes from the start. A number of investigations of swine abortion have been published since 1916. From the material there presented, two problems appear; first, the significance of the porcine race in the abortion disease of cattle and the reverse, and second, the nature of the guinea pig disease induced by the porcine race. Taking up the published experiments on the first problem, the identity of bovine and porcine races, we find the following data at hand.

Hadley and Beach (19) produced abortion in a pregnant cow and in sows with the porcine strain. A bovine strain caused abortion in a pregnant cow, but not in sows. Cotton (20) failed to infect pregnant sows with bovine strains by feeding. Porcine strains failed in two pregnant cows after intravenous injection. In another cow treated similarly, premature birth followed. The fetus and placenta were infected.

Huddleson (21) fed three virgin pigs with naturally infected milk and virulent cultures of bovine origin, beginning when pigs were 8 weeks old and continuing to time of farrowing, without producing abortion. Four pigs placed with infected cows remained normal. Six fed large doses both of bovine and porcine cultures repeatedly, dropped normal litters.

Schroeder and Cotton (22) produced abortion in pregnant cows by intravenous injection of swine abortion cultures, but not by feeding or cohabitation of swine-infected cows with other cows. Sows were not infected by feeding bovine strains.

Before dismissing these experiments of direct feeding or inoculation of cattle and pigs with homologous and heterologous strains, it may be stated that intravenous injection of pregnant animals may

[1] Quoted by Hayes (23).

break down a resistance sufficient to prevent infection through natural agencies. On the whole the experiments quoted are not conclusive. They indicate either that the porcine type of bacillus is harmless to cattle and the reverse, or else that the porcine type is of higher virulence and may infect cattle.

The effect of *B. abortus* from swine on guinea pigs is mentioned by several writers.

Hayes (23) states that "porcine strains proved, on the whole, to be more virulent for guinea pigs, causing, with a few exceptions, involvement of the testes and one or both radiocarpal regions, also general adenitis. Thirteen of twenty-two guinea pigs inoculated with porcine strains succumbed within 2 months, while none of the twelve inoculated with the bovine strain died of infection during that period."

Cotton (20) noticed differences between the lesions in guinea pigs produced by the swine abortion strain and the bovine strains. The lymph nodes are "converted into sacs filled with semi-fluid, necrotic material." He also describes in the spleen, "spherical nodules varying in size from 1 to 7 mm. in diameter" filled with odorless necrotic material. Similar nodules were found in the liver. A frequent lesion produced by another strain was an abscess of the orbit behind or below the eyeball. Cotton studied strains from Missouri and California and found the same type of lesion in all.

Weeter (24), in attempts to isolate the porcine strain from the uteri of swine, obtained cultures three times in 259 non-gravid uteri and once in 181 gravid uteri. Following the inoculation of guinea pigs, "two of the strains produced more lesions of the spleen than were given by many of the bovine strains, but these lesions were not more marked than were obtained with a freshly isolated culture from bovine sources."

Schroeder and Cotton (25) find that after placing 1 drop of a heavy suspension of *B. abortus* on the conjunctiva of guinea pigs there was 33 ⅓ per cent infection following the bovine type and 100 per cent following the porcine type. Placing 1 drop on tongue, 58 ⅓ per cent of porcine and none of bovine type infection resulted. Of twelve controls placed with these, none of bovine controls, but seven of porcine controls became infected. In these experiments it is not stated what constitutes infection.

Outbreaks of swine abortion appear to be restricted thus far to the Middle West and the western coast. Fresh cultures were, therefore, not obtainable for a renewed study of this type of *B. abortus*. In view of the practical importance of the subject, five strains received from outside sources were tested on guinea pigs. The meager but significant outcome may be briefly summarized.

Two strains were obtained in 1923, which had been isolated in

Missouri. These were tested on guinea pigs under the same conditions and at the same time that *B. melitensis* II and III were under observation. Four guinea pigs were inoculated from each strain. The animals were killed in the 3rd month following inoculation. In three animals inoculated from strain designated I, there were no lesions observable and cultures made with the entire spleen were negative. The fourth animal developed a bulging swelling on the inner aspect of the left knee joint which became centrally necrotic. Both thymus glands grew to twice the normal size and were filled with abscesses 4 to 5 mm. in diameter. The epididymis of right testicle was shrunken but not abscessed. *B. abortus* developed in all of three spleen cultures.

The four guinea pigs inoculated with the second strain were killed at the same time. None presented any enlarged spleen or diseased testicles, nor were lymph nodes appreciably swollen. From three, however, *B. abortus* was recovered from the spleen where it was present in very small numbers.

Recently the writer received, through the kindness of Dr. E. C. Schroeder, three strains of the porcine variety, which are designated below as III, IV, and V. III and V were isolated in Illinois, IV in Minnesota. Their total age in artificial media was not communicated. They grew luxuriantly in unsealed tubes. Four guinea pigs were inoculated with each strain. The dose used was slightly heavier than in preceding tests. 1 cc. of a $\frac{1}{500,000}$ dilution of the condensation water after washing the growth into it was injected subcutaneously in the left flank. The guinea pigs were killed between 2 ½ and 3 months following inoculation.

The results indicated marked attenuation. In one guinea pig receiving Strain III, the left kneefold node contained a 5 mm. abscess. In the liver were eight to ten similar softened foci from 2 to 10 mm. in diameter. The spleen was small. In a second animal, yellow, soft foci occurred in the left kneefold nodes, in the right axillary, the submental, the cervical, and the left pelvic node. These various foci or abscesses were up to 4 mm. in diameter. The small spleen contained two 1 mm. foci. In the third animal, only the kneefold node nearest the site of inoculation had two softened foci. The fourth animal was negative. Spleen cultures remained sterile. The abscesses usually contained living bacilli.

In three of the guinea pigs receiving Strain IV, one or two small abscesses were found in the left kneefold nodes. The fourth animal was entirely negative. Cultures of spleen tissue were negative.

In one of the four guinea pigs receiving Strain V there were two small foci in the left kneefold node. In a second guinea pig, both left kneefold and one cervical node contained a centrally cheesy focus, 1.5 mm. in diameter. The two remaining animals were entirely normal. All spleen cultures remained sterile.

The results thus obtained from the five strains indicate a great decline in virulence if we take the published statements as a basis for our judgment.

DISCUSSION.

The impression first made upon the writer, accustomed to the bovine disease, by the macroscopic appearance of the spleen, lymph nodes, and thymus of the guinea pigs inoculated with the human strains was that the disease was entirely distinct from that produced by *B. abortus*. This superficial impression was quickly reduced to proper dimensions by further study. The uniformity of the picture due to the bovine races contrasted with the quite different uniformity of lesions produced by the two human strains, isolated at different times in different sections of the country, furnishes strong evidence that the two types are not identical. The differences between them are obviously not great and in themselves supply evidence of the fundamental relationship of the bovine and the human strains. The question before us is not, however, the closeness of this relationship but the capacity of the bovine race to invade the human subject and multiply enough to produce symptoms characteristic of Malta fever.

The histological character of the focal lesions produced by the two races in guinea pigs may be briefly summed up as follows: Both produce in spleen and lymph nodes a proliferation of a certain type of cell which, for convenience, is called endothelial. It resembles the cells focalized by the tubercle bacillus without, however, undergoing the same retrogressive changes. The cells retain stains feebly and thus give the impression of rarefied areas under low powers. In the *B. abortus* animals the proliferation is not extensive. The groups

of cells are small, ranging from a few cells to masses 0.4 to 0.6 mm. in diameter. Frequently the groups coalesce into a broad meshwork throughout the organ. The amount of proliferation varies with the strain and age of the lesion. In very rare instances when the cell focus attains the maximum dimensions given, a nucleus of polymorphs may occupy the center. Necrosis has not been observed in any animal inoculated with early cultures. In only one case of many hundreds a small softened focus was found in the kneefold node of a guinea pig inoculated directly with uterine washings. In such cases it is rather remarkable that more cases do not occur in view of the rapid invasion of the postparturient uterus by cocci and bacilli of various kinds which are injected with *B. abortus* in the washings.

In the guinea pigs receiving the human strains the primary lesion is identical with that of the bovine disease. There is proliferation of the same cell type at the start. After the 2nd or 3rd week, some or all of the cell foci enlarge until they become visible to the naked eye as yellow masses 2 to 3 mm. in diameter, sharply outlined but not raised. Sections show extensive polymorph infiltration with or without central necrosis. When lymph nodes and thymus have enlarged to 1 cm. in diameter, it is probable that many foci have coalesced. The entire gland is then converted into a stiff mass enclosed in a thin-walled capsule.

The polymorph invasion and necrosis appear to run parallel with the size of the endothelial foci. Small foci remain unchanged. In the testicles the foci resulting from the two types ultimately suppurate. In the *B. abortus* animals this secondary change may be tentatively referred to the presence of masses of spermatozoa in the tubules which become imprisoned on account of the pressure of the interstitial foci. The compressed tubules have been observed to become centers of necrosis. In organs closed to the exterior, such as spleen and lymph nodes, polymorph invasion leading to softening has not been observed in *B. abortus* animals.

The injection of large numbers of *B. abortus* may lead locally to suppurative processes due to the presence of intracellular bacterial poisons. In the animals given minute doses, the early process is always an endothelial proliferation in lymph nodes and spleen. In-

jection into the peritoneal cavity and the subcutis produced the same type of disease, if we except a slight difference in the course of the lymph node lesions due to the original site of deposit of the bacteria. Following the intraperitoneal route, the testicles were somewhat earlier and more severely involved in both groups of guinea pigs.

The turn in the cellular reaction of guinea pigs infected with the human strains leading to a suppurative infiltration of the primary foci of endothelial elements suggested the idea that perhaps the blood picture of inoculated guinea pigs might reflect this turn and make a differential diagnosis between *B. abortus* and the human strains feasible. To gauge this possibility, Miss M. L. Orcutt studied the total and the differential count of the leucocytes of guinea pigs inoculated with the two strains of *B. abortus* (1114 and 1119) and the two strains of *B. melitensis*. To these were added two control animals. The counts were made once a week for 13 weeks. The results were disappointing in so far as they did not present differences wide enough for building on them any differential diagnosis between the *B. abortus*, the human, and the control guinea pigs.

To meet the possible objection that *B. abortus* may produce lesions similar to *B. melitensis* after it has been cultured for some time, six strains of *B. abortus* from five different States and originally isolated by the writer were injected into guinea pigs in doses corresponding to those used throughout in this study. The animals were killed after 2 and 3 months, respectively. In no case were lesions found undergoing suppurative changes. The decline in virulence resulted simply in a fading out of the lesions without a change in the character of the reaction. One strain of *B. abortus* isolated 6½ years ago had become avirulent. Two guinea pigs killed 63 and 83 days after inoculation, respectively, failed to show any lesions and cultures of the entire spleen were negative. The weight of the guinea pigs increased during this time from 405 and 410 gm. to 725 and 830 gm., respectively. Three strains from three different herds isolated 5 years ago still produced moderately enlarged spleens and localizations in one or both testicles. Spleen cultures were positive. One strain isolated 2 years, and another 15 months ago were less virulent than the preceding.

It is fairly obvious from the results obtained that strains which

have a long period of artificial culture behind them are not adapted for the determination of subtle differences, since the lesions produced tend to converge in a negative direction. This is emphasized by results obtained with a strain of *B. melitensis* isolated by Dr. Zammit from goat's milk on the Island of Malta in 1920. This strain, kindly sent by the Lister Institute, was inoculated into guinea pigs in the same doses used on *B. melitensis* II and III. Six guinea pigs were used. After inoculation the weights continued to increase. Two were killed after 27 days, two after 34 days, and two after 42 days. In none was there any spleen enlargement or other lesion. The entire spleens of four were cultured by tearing each into several bits and transferring to agar slants. In only one a growth appeared above the bit of spleen in the condensation water. This was identified as the organism inoculated.

Turning to the porcine race of *B. abortus* we find both in the published statements quoted and in the results of the guinea pig inoculations outlined above that this race resembles the two human strains much more closely than do the bovine strains. Whenever any lesions were produced they resembled those following inoculation with the human strains. We must, therefore, be prepared to look for the source of the human infections in the bacillus of swine abortion, provided the caprine type is not in evidence. The fact that Dr. Blake's patient was occupied in a slaughter house with handling fresh pork lends support to this view.[2]

When races resemble one another as closely as do the three strains of *B. abortus* there may be an occasional aberrant infection of one host with the strain from another host, as is true of the three races of tubercle bacilli. The close association of cattle and swine or of goats and cattle may lead to the infection of some abnormally susceptible member of one host species with the parasite of the other host. On the whole, however, such an event is probably rare.

A renewed thorough investigation of the effects of *B. melitensis* from the goat on guinea pigs is called for before the differential characters of the three primary races of this species can be clearly established. Slightly modified, intermediate types due to occasional

[2] Personal communication.

aberrant parasitism are to be anticipated. In the meantime, the evidence that *B. abortus* producing disease of the placenta in cattle may produce a disease in man simulating Malta fever must be regarded as inadequate in establishing any such relationship. The situation as it presents itself is very much like the one forced upon bacteriology in 1898 with reference to the tubercle bacillus. The problem is a thorough study of individual strains found in the human subject together with further investigations of the porcine strains.

A study of the agglutination affinities of the strains described in this paper was made by M. L. Orcutt (26). It will be seen by referring to her paper that with the technique used by her all strains acted alike.

CONCLUSIONS.

1. Comparative tests on guinea pigs with *B. abortus* yield the best results when minute doses are injected subcutaneously and the animal kept at least 4 weeks.

2. *B. abortus* gradually loses its virulence for guinea pigs under artificial cultivation.

3. Two cultures resembling *B. abortus* from cattle, isolated from human cases of so called Malta fever, are shown to be in their effect on guinea pigs not identical with the bovine strains.

4. The results of studies of *B. abortus* from swine indicate a close relation between the porcine strains and the two human strains.

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AGGLUTINATION AFFINITIES OF THE ABORTUS-MELITENSIS GROUP OF BACTERIA WITH SPECIAL REFERENCE TO TWO HUMAN STRAINS.

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In view of the differences between *B. abortus* of bovine origin and two human strains resembling *B. melitensis* described by T. Smith,[1] it seemed desirable to examine the serological relations of the strains compared by him with especial reference to agglutination.

According to Evans' first paper,[2] a *B. abortus* antiserum agglutinated both types of cultures alike, but a *melitensis* antiserum agglutinated *B. melitensis* to a higher titer than *B. abortus*. In absorption experiments there was little difference in the reactions with the *abortus* antiserum. The *melitensis* antiserum treated with *B. melitensis* lost all agglutinins for both cultures, but absorption with *B. abortus* removed only agglutinins for itself and left agglutinins for *B. melitensis* still present to a considerable degree.

Feusier and Meyer[3] made a serological study of fourteen strains. A series of agglutinin absorption tests resulted in the separation of the strains into four groups.

In 1923 Evans[4] reported on the serological classification of 49 strains from human, bovine, caprine, porcine, and equine sources, obtained from different parts of the world. These included strains from Groups 1, 2, and 3 of Meyer and Feusier. Altogether seven groups were created, three main groups and four smaller subgroups. More recently Evans[5] described eight serological groups. The only known method by which *B. abortus* and *B. melitensis* strains can be differentiated, according to Evans, is by the agglutinin absorption test, and by this method the differentiation is slight for saturation of a serum of either type with an antigen of the heterologous type will remove from 85 to 90 per cent of the

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[4] Evans, A. C., *Pub. Health Rep.*, 1923, xxxviii, 1948.

[5] Evans, A. C., *Abstr. Bact.*, 1925, ix, 23.

agglutinins for the homologous antigen. Furthermore she found that the strains from Mediterranean countries showed a serological grouping distinct from strains from northern countries.

Zeller[6] obtained eight strains of *B. melitensis* from various institutes and compared them with strains of *B. abortus* which he had isolated himself. Sera obtained by immunizing rabbits with *B. abortus* and *B. melitensis* agglutinated both groups of cultures alike. He also found that after reciprocal absorptions of the sera by *B. abortus* and *B. melitensis* he could ascertain no differences between the two kinds of bacteria.

Skarić[7] worked with four *B. melitensis* strains and eight *B. abortus* cultures. Each type of serum agglutinated its own variety of culture to a higher titer than the other type and this was more marked with the *abortus* antiserum than with the *melitensis* antiserum. In the agglutinin absorption experiments only the homologous strain completely absorbed all agglutinins for all strains. The chief differentiation occurred by absorption of the *melitensis* antiserum with *B. abortus* cultures which completely removed agglutinins for *B. abortus* but only partially absorbed agglutinins for *B. melitensis*.

Khaled[8] made a comparative study of thirty strains of *B. abortus* and *B. melitensis*. He found that his *melitensis* antiserum agglutinated both sets of cultures alike, but the *abortus* antiserum agglutinated the *abortus* cultures to a slightly higher titer than the *melitensis* strains. His absorption results showed a distinction only when the *abortus* antiserum was absorbed by *B. melitensis*. This removed agglutinins for *B. melitensis* but not for *B. abortus*.

Burnet[9] states that a *melitensis* antiserum obtained either from a patient or from a goat or from treated rabbits or guinea pigs agglutinated *B. abortus* and *B. melitensis* cultures alike or *B. abortus* to a slightly higher titer. On the other hand, *B. abortus* antisera prepared from guinea pigs or rabbits always agglutinated *B. abortus* to a higher titer than *B. melitensis*. Thus he concluded that the *abortus* strains in general were composed of a more comprehensive antigen than *B. melitensis*. He also states that whatever the differences established by agglutination tests between *B. melitensis* and *B. abortus*, it is certain that one will find also well marked differences between authentic strains of *B. melitensis*; and if all the attempts at grouping have any significance, they only tend to establish relative distinctions between representatives of the same species.

Futamura[10] studied twenty strains of *B. abortus* isolated from cattle in Japan and compared these with two strains of *B. melitensis* and one strain of swine *B. abortus* from England. His results showed that antisera prepared from each type of culture gave an identical agglutination titer for each type of organism

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[6] Zeller, H., *Berl. tierärztl. Woch.*, 1920, xxxvi, 345.

[7] Skarić, J., *Z. Hyg. u. Infectionskrankh.*, 1922, xcv, 358.

[8] Khaled, Z., *J. Hyg.*, 1921, xx, 319.

[9] Burnet, Et., *Arch. Inst. Pasteur Afrique Nord*, 1923, iii, 48.

[10] Futamura, H., *J. Jap. Soc. Vet. Sc.*, 1924, iii, 145.

when living cultures were used, but if the antigen was heated at 60°C. or 100°C. for an hour the *B. abortus* antiserum agglutinated *B. abortus* cultures more powerfully than *B. melitensis*. The results of absorption tests showed that the *abortus* and *melitensis* antisera contained 88 to 89 per cent of common agglutinins and 11 to 12 per cent of specific agglutinins. The common agglutinogens and those specific for *B. abortus* were thermostable and the specific *melitensis* agglutinogens were thermolabile. He found no serological difference between the bovine and swine strains.

Ficai and Alessandrini[11] found that a *melitensis* antiserum unheated agglutinated both *melitensis* and *abortus* cultures, but if the serum was heated at 60°C. it still agglutinated the *melitensis* culture but failed to agglutinate the *abortus* culture. On the other hand, an *abortus* antiserum unheated agglutinated both cultures, but heated at 65°C. it still agglutinated *B. abortus* cultures and failed to agglutinate *melitensis* cultures. A *melitensis* antiserum heated at 65°C. did not agglutinate either culture, while a *B. abortus* antiserum heated at this temperature still agglutinated *B. abortus* to the original titer and heated at 75°C. still gave a definite reaction.

In publications comparing *B. abortus* of bovine and swine origin there is agreement on their serological identity. Doyle and Spray[12] stated that the swine and bovine strains were alike serologically. Hayes[13] in some studies on swine abortion mentioned that absorption tests with sera from rabbits immunized against three hog strains and against two bovine strains and sera from guinea pigs immunized against one bovine strain and serum from a naturally infected cow showed no differences. Cotton[14] studied six strains of *B. abortus* obtained from abortion disease of swine occurring in various regions. All six of the swine strains were like the bovine strains serologically.

EXPERIMENTAL DATA.

The agglutination and agglutinin absorption experiments to be reported here were made with six strains designated *B. abortus* 1114, and *B. abortus* 1119 (of bovine origin); *B. melitensis* II (Blake), and *B. melitensis* III (Baltimore) (from human cases); and *B. abortus* Swine I, and *B. abortus* Swine II. For further data on these cultures the reader is referred to Smith's paper.[1]

Sera were prepared by immunizing rabbits with *B. abortus* 1119, *B. melitensis* II, *B. melitensis* III, and *B. abortus* Swine I cultures all heated at 60°C. for ½ hour.

[11] Ficai, G., and Alessandrini, A., *Ann. ig.*, 1925, xxxv, 1.
[12] Doyle, L. P., and Spray, R. S., *J. Infect. Dis.*, 1920, xxvii, 165.
[13] Hayes, F., *J. Am. Vet. Med. Assn.*, 1921–22, lx, N.S. xiii, 435.
[14] Cotton, W. E., *J. Am. Vet. Med. Assn.*, 1922–23, lxii, N.S. xv, 179.

Complete series of agglutination and cross-agglutination tests were carried out and each serum was absorbed by all the cultures and then tested for agglutinins against each strain. In general the method of Feusier and Meyer was followed in the absorption procedure. The serum was diluted 1/5 or 1/10 with physiological salt solution. The culture was grown on agar and suspended in salt solution. Equal volumes of the diluted serum and culture suspension were mixed and incubated at 37°C. for 2 hours and then refrigerated overnight. The mixture was centrifuged the next day and the clear serum tested with the absorbing strain for complete absorption. If agglutinins still remained for the absorbing strain, then more culture was added to complete the absorption before testing the serum against the other strains.

The results showed that each serum agglutinated all the strains equally, and after absorption according to the above method each strain removed all agglutinins for the homologous strain as well as for the other strains. The tabulated results of a complete agglutination and absorption experiment with one serum will illustrate the reactions obtained in all cases.

All the cultures absorbed all agglutinins leaving none for homologous or heterologous strains. This was the case with each lot of serum. These results indicate that these strains cannot be separated or distinguished by agglutination and absorption tests carried out as described above. *B. abortus* 1114 and *B. abortus* 1119 are, however, known to be bovine strains. The fact that the two swine strains, S.I and S.II, acted like the bovine cultures is in agreement with other workers who state that bovine and swine strains of *B. abortus* are alike serologically. Since the strains *B. melitensis* II and *B. melitensis* III were identical in their serological behavior with *B. abortus* 1119 and *B. abortus* 1114 they may be classed as the bovine type serologically.

In making experiments with heated sera according to the methods of Ficai and Alessandrini we found that the *B. abortus* 1119 antiserum after having been heated at 65°C. for ½ hour still agglutinated all the cultures to the original titer. Therefore, according to Ficai and Alessandrini, the *B. melitensis* II and *B. melitensis* III strains behaved like bovine type cultures in heated *abortus* antiserum. The heating experiments were made also with *B. melitensis* II and *B. melitensis* III antisera. Here again after heating at 60°C. for ½ hour these sera still agglutinated both the *B. abortus* and *B.*

TABLE I.

B. abortus 1119 (Bovine) Antiserum.

	Agglutinated by	Dilutions. 1:40	1:80	1:160	1:320	1:640	1:1,280
Agglutination titer with homologous strain (B. ab. 1119).							
Before absorption.	B. ab. 1119	C.	C.	C.	C.	++++	+
After absorption by							
B. ab. 1119.....	"	—	—	—	—	—	—
" 1114.....	"	—	—	—	—	—	—
B. melit. II.....	"	—	—	—	—	—	—
" III.....	"	—	—	—	—	—	—
B. ab. S. I.....	"	—	—	—	—	—	—
" " II.....	"	—	—	—	—	—	—
Agglutination titer with non-homologous strains.							
Before absorption.	B. ab. 1114	C.	C.	C.	C.	+++	+
	B. melit. II	C.	C.	C.	C.	++	+
	" III	C.	C.	C.	C.	+++	+
	B. ab. S. I	C.	C.	++++	++++	+++	++
	" " II	C.	C.	++++	++++	++	+
After absorption by							
B. ab. 1119.....	B. ab. 1114	—	—	—	—	—	—
" 1114.....	"	—	—	—	—	—	—
B. melit. II.....	"	—	—	—	—	—	—
" III.....	"	—	—	—	—	—	—
B. ab. S. I.....	"	—	—	—	—	—	—
" " II.....	"	—	—	—	—	—	—
B. ab. 1119.....	B. melit. II	—	—	—	—	—	—
" 1114.....	"	—	—	—	—	—	—
B. melit. II.....	"	—	—	—	—	—	—
" III.....	"	—	—	—	—	—	—
B. ab. S. I.....	"	—	—	—	—	—	—
" " II.....	"	—	—	—	—	—	—
B. ab. 1119.....	B. melit. III	—	—	—	—	—	—
" 1114.....	"	—	—	—	—	—	—
B. melit. II.....	"	—	—	—	—	—	—
" III.....	"	—	—	—	—	—	—
B. ab. S. I.....	"	—	—	—	—	—	—
" " II.....	"	—	—	—	—	—	—

TABLE I—*Concluded.*

	Agglutinated by	Dilutions.					
		1:40	1:80	1:160	1:320	1:640	1:1,280
After absorption by							
B. ab. 1119.....	B. ab. S. I	—	—	—	—	—	—
" 1114.....	"	—	—	—	—	—	—
B. melit. II.....	"	—	—	—	—	—	—
" III.....	"	—	—	—	—	—	—
B. ab. S. I.....	"	—	—	—	—	—	—
" " II.....	"	—	—	—	—	—	—
B. ab. 1119.....	B. ab. S. II	—	—	—	—	—	—
" 1114.....	"	—	—	—	—	—	—
B. melit. II.....	"	—	—	—	—	—	—
" III.....	"	—	—	—	—	—	—
B. ab. S. I.....	"	—	—	—	—	—	—
" " II.....	"	—	—	—	—	—	—

melitensis cultures to the original titer, but in the lower dilutions of the heated *B. melitensis* III antiserum a zone of inhibition occurred for all cultures. Since the *B. abortus* strains (1114 and 1119) were agglutinated to the original titer in the heated *B. melitensis* II and *B. melitensis* III antisera, then, according to Ficai and Alessandrini, these sera behaved like *B. abortus* antisera. In the previous test it was seen that strains *B. melitensis* II and III behaved like *abortus* cultures in the inactivated *abortus* antiserum. Therefore, if Ficai and Alessandrini's method of distinguishing *B. abortus* and *B. melitensis* agglutinins by heat is correct, these results are further evidence for placing strains *B. melitensis* II and III serologically with the *abortus* type of organism. Heating *B. abortus* Swine I antiserum gave results corresponding to the bovine type, but a marked zone of inhibition for all cultures occurred in the lower dilutions. Since no differences occurred at the temperatures of 60°C. and 65°C., found critical by Ficai and Alessandrini, a higher temperature of 75°C. was tried. *B. abortus* 1119, *B. melitensis* II and III, and *B. abortus* Swine I antisera heated at 75°C. for ½ hour gave no agglutination with any of the six cultures. Thus in all experiments with heated sera all the strains behaved alike.

TABLE II.

B. melitensis II Antiserum.

Culture.	Unheated.						Heated (60°C.—1/2 hr.).					
	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:80	1:160	1:320	1:640	1:1,280	1:2,560
B. melit. II unheated	C.	C.	C.	C.	++++	++	C.	C.	C.	C.	C.	++
" II heated 100°C.—2 hrs.	C.	C.	C.	C.	++++	++	C.	C.	++++	++++	+++	+
B. ab. 1119 unheated	C.	C.	C.	C.	++++	++	C.	C.	C.	C.	C.	++
" 1119 heated 100°C.—2 hrs.	C.	C.	C.	C.	++++	++	C.	++++	++++	+++	++	+

B. abortus 1119 Antiserum.

Culture.	Unheated.						Heated (65°C.—1/2 hr.).					
B. melit. II unheated	C.	C.	C.	++	±	−	++++	C.	C.	C.	+++	+
" II heated 100°C.—2 hrs.	C.	C.	++++	++	+	−	++++	++++	+++	++	+	+
B. ab. 1119 unheated	C.	C.	C.	++++	+	−	++++	C.	C.	C.	+++	+
" 1119 heated 100°C.—2 hrs.	C.	C.	++++	++	+	−	++++	+++	+++	+++	++	+

Futamura used heated antigens and claimed a difference in reaction in the *abortus* antiserum. He also stated that specific *melitensis* antigen was heat-labile and specific *abortus* antigen and the common antigen were heat-stable. This would mean that a *melitensis* culture after having been heated would give a lower agglutination titer, and if such a culture was combined with a heated *melitensis* serum which contained only specific *melitensis* agglutinins (Ficai and Alessandrini), then the titer would be negative. With these points in mind some experiments were made with heated and unheated cultures in heated and unheated sera. The results with two strains are given in Table II.

The results showed that the agglutinins and agglutinogens of all the cultures were heat-stable at the given temperatures. There was no evidence of a heat-labile specific *melitensis* antigen in the strains *B. melitensis* II and *B. melitensis* III.

CONCLUSIONS.

The conclusions to be drawn from this study are that while there appears to be evidence of serological distinctions between *B. abortus* and *B. melitensis* cultures as studied by earlier workers, the experiments reported in this paper show no serological distinctions among the six strains used in the study. *B. melitensis* II and *B. melitensis* III were found to be identical with the bovine and swine strains by means of agglutination and agglutinin absorption reactions in unheated sera, by agglutination in heated sera, and with the use of heated cultures in unheated and heated sera.

THE ORIGIN OF THE LEPRA CELL.

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PLATE 4.

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One of the most striking features of the anatomical changes which develop in leprosy is the occurrence in the lesion of great numbers of large phagocytic cells filled with bacilli. These cells were first noted by Virchow,[1] and were given the name of "lepra cells" by him. Later investigation has shown that they are characteristic of the disease, the leprous nodule being composed almost entirely of them with a varying admixture of fibroblasts and plasma cells or lymphocytes. In this regard at least they may be considered analogous to the epithelioid cell of the tubercle.

The morphology of these cells is remarkably constant. They vary in size from that of a lymphocyte to several times this diameter. Their protoplasm, which forms the greater part of the cell, is filled with vacuoles, and contains enormous numbers of lepra bacilli. The nucleus is usually single and is as a rule pressed to one side by the vacuoles and bacilli that crowd the cell body.

There is still considerable controversy as to the origin of these cells.

Some have even denied that they are cells but interpret them as lymphatics plugged with bacilli or clumps of bacilli embedded in a jelly-like substance. According to Neisser[2] and Marchoux[3] they are of hematogenous origin, and are derived from lymphocytes which have wandered out into the infected tissues from the circulating blood. They therefore consider them examples of Metchnikoff's macrophages. Baumgarten[4] and Philippson,[5] on the other hand, describe

[1] Virchow, R., Die krankhaften Geschwülste, 1864–65, ii, 514 *et seq.*

[2] Neisser, A., *Virchows Arch. path. Anat.*, 1881, lxxxiv, 514.

[3] Marchoux, E., and Sorel, F., *Ann. Inst. Pasteur*, 1912, xxvi, 675.

[4] Baumgarten, P., *Berl. klin. Woch.*, 1888, xxv, 217.

[5] Philippson, L., *Virchows Arch. path. Anat.*, 1893, cxxxii, 229.

them as originating from the fibroblasts and fixed connective tissue cells of the tissue involved. The problem in regard to their histogenesis is therefore the same as exists with regard to the analogous epithelioid cells of the tubercle. Purely morphological study led to no certain decision as to the origin of these cells and it was only the introduction of a method of functional differentiation by means of vital staining that objective evidence was obtained. By this means Oppenheimer,[6] Goldmann,[7] and others showed conclusively that the epithelioid cells of the tubercle are derived at least in part from certain tissue cells; namely, from the histiocyte or reticulo-endothelial cells.

Although the inoculation of animals with lepra bacilli produces only doubtful reactions, material for an experimental study of the question is available in the spontaneous leprosy that occurs in rats. The relation between the two diseases, that of man and that of the rat, is certainly a very close one. The microscopic appearance of the lesions is in all essentials identical, the lepra cells in particular being equally well developed in the infection of the rat as in that of man. Moreover, several investigators, Mezincescu,[8] Schmitt,[9] and Bayon,[10] have found evidence by means of complement fixation tests which indicates a close relationship between the organisms found in the two diseases. Bayon, in fact, considers them to be identical.

Through the kindness of Dr. N. E. Wayson, of the United States Public Health Service, we obtained a wild rat in which the disease was well developed and have applied the method of vital staining to a study of the origin of the lepra cells in animals inoculated from this source.

Methods.

Six white rats were inoculated intraperitoneally May 23, 1924, with an emulsion of the subcutaneous leprous infiltrations from the spontaneous case. Examination had shown the material to be loaded with acid-fast bacilli. Two of the animals died of intercurrent infections during the next few months, and the remaining four showed no external evidence of the disease after 6 months. At 10 months two rats were given a subcutaneous injection of 2 cc. of 1 per cent aqueous trypan blue and the injection was repeated twice at intervals of 6 days. At the end of

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[6] Oppenheimer, R., *Virchows Arch. path. Anat.*, 1908, cxciv, suppl., 254.

[7] Goldmann, E., Neue Untersuchungen über die äussere and innere Sekretion des gesunden und kranken Organismus im Lichte der "vitalen Färbung," Tübingen, 1912, 67.

[8] Mezincescu, D., *Compt. rend. Soc. biol.*, 1908, lxiv, 514.

[9] Schmitt, L. S., *Univ. Calif. Pub. Path.*, 1911, ii, 29.

[10] Bayon, H., *Brit. Med. J.*, 1912, ii, 1191.

the experiment they were stained a deep blue but otherwise appeared normal. They were then killed and fixed *in toto* in 10 per cent neutral formalin.

Dissection of the animals showed in the inguinal regions and extending over the lower abdomen a diffuse infiltration with soft granulation tissue. Smears of this tissue showed enormous numbers of acid-fast bacilli. No lesions were noted in the internal viscera.

The remaining two rats were stained in the same way 11 months following their inoculation and were then killed. The lesions found in them were of the same nature as in the earlier experiments except that the process was rather more widespread in the subcutaneous tissue and that small nodules were found in the liver and lungs of one animal. Material from the different animals was then embedded in paraffin and sections stained with carmine, hematoxylin and eosin, Van Gieson's mixture, the Giemsa method, and with carbolfuchsin for the bacilli.

EXPERIMENTAL FINDINGS.

The lesions found in all the animals are substantially the same and for this reason only a general description of them is given. The essential process in all the tissues is the development of nodules of lepra cells followed by a late necrosis. These nodules are of the same character and the manner of their formation is the same whether in the subcutaneous tissues or in such internal organs as the liver and lungs. The nodule consists of an accumulation of lepra cells (Fig. 1). These are large oval cells, except where they are compressed by crowding together, whose abundant protoplasm is filled with round vacuoles. In some instances these vacuoles are so numerous that there is little actual protoplasm remaining, the cell body having the appearance of a foam. The nucleus of the cell is small and oval and is compressed and displaced to one side by the vacuoles which crowd the cell body. If the section has been stained with carbolfuchsin and decolorized with acid alcohol the significance of the vacuolization and distension of the protoplasm is seen. The lepra cell is so filled with acid-fast bacilli that all details, except the compressed nucleus, are obscured. The whole nodule appears to be made up of clusters of bacilli and it is only with some difficulty that the cellular nature of the individual clumps becomes evident. Not all the bacilli are intracellular however. Many lie free in the tissue, liberated apparently by the rupture of the distended lepra cells. These in turn are taken up by phagocytic cells and as intracellular proliferation of them progresses this

phagocyte is gradually transformed into the typical lepra cell and forms part of the nodule.

This process of growth of the nodule by the approximation of new cells can be quite clearly followed at its periphery and there the genesis of the lepra cell is clearly evident in the vitally stained animal.

In the subcutaneous lesions the nodules lie scattered through the loose alveolar connective tissue that lies over the fascia of the muscle. On examining this tissue one is immediately struck by the tremendous number of vitally stained histiocytes that are present (Fig. 1). In normal subcutaneous tissue these cells are present only in moderate numbers, and are scattered rather widely among the more numerous fibroblasts, but in the infected tissues the ratio between ordinary fixed tissue cells and histiocytes is reversed, there being relatively more of the latter. Even at some distance from the site of the lesion this increase in the number of the vitally stained cells is quite evident. The purpose of this mobilization of the histiocytes is evident when one studies the transformation which occurs in these cells as one approaches the focus of infection; *i.e.*, the leprous nodule.

At some distance from the nodule the histiocytes show their typical morphology. They possess a well developed oval nucleus and a moderate amount of protoplasm which, on account of the mobility of these cells, is of irregular shape and which is crowded with blue dye granules. As one approaches the focus of infection one finds scattered among the normal histiocytes an occasional cell which contains fewer dye granules and whose protoplasm is swollen. Still nearer to the nodule, cells are found which contain only an occasional granule of blue and in their distended protoplasm vacuoles are present. In the immediate periphery of the mass of typical lepra cells the majority of the histiocytes have but one or two dye granules, while the greater part of their swollen protoplasmic body has all the characteristics of the typical lepra cells. There is, in other words, a gradual transformation of the histiocyte into the lepra cell.

In sections stained with carbolfuchsin one finds the bacilli in the vitally stained histiocytes (Fig. 2). There is a rough inverse variation between the number of dye granules and the number of bacilli in the protoplasmic bodies of the cells. If the cell contains many bacilli there are few granules, and those cells which contain their normal

amount of dye show only an occasional bacillus. At some distance from the nodule one finds the histiocytes therefore free of bacilli and presenting their usual deeply stained appearances. As one approaches the leprous nodule the number of phagocytosed bacilli increases, the protoplasm of the cells swells and becomes vacuolar as previously described, and the dye granules gradually disappear until, in the completely formed lepra cell of the nodule, the protoplasm contains a mass of bacilli only and no dye (Fig. 2). The other types of cells which lie around the lepra nodules and which, with proliferating capillaries, form a granulation tissue in which the histiocytes lie, take little or no part in the formation of the lepra cells. These cells are fibroblasts and round cells such as lymphocytes and plasma cells. The fibroblasts, easily identified by their morphology and lack of dye granules, though they may proliferate and form more or less collagen between and around the nodules, do not undergo any changes which could produce the swollen vacuolar lepra cells. Occasionally one sees such a cell containing a bacillus, but they remain passive and do not react to its presence. Nor do the lymphocytes and plasma cells either contain the bacilli in great numbers or are they transformed to the very different type of cell represented by the lepra cells.

In short, though the leprous nodule may contain fibroblasts and round cells, its typical cellular component, the lepra cells, is derived from the histiocyte alone. In other organs, such as the liver and lung, a similar process may be observed. In the liver it is the vitally stained von Kupffer cells which proliferate and collect around the periphery of the nodule, phagocytose the bacilli, and ultimately lose their dye granules and become typical lepra cells. In the lungs the nodules are found in the interstitial tissue, especially around the bronchi and blood vessels. Here vitally stained histiocytes are found in increased number and the same transformation of the heavily stained bacillus-free histiocyte to the bacillus-laden dye-free swollen vacuolar lepra cell is seen.

DISCUSSION.

The lepra cells when studied by means of vital staining are found to be derived from the histiocyte of the infected tissue. Fibroblasts and lymphocytes play an insignificant part in their formation although

they may be present as such in the nodule or in the granulation tissue which forms about the bacilli. If not stained vitally the histiocyte is, on account of its varying size and shape, an exceedingly difficult cell to identify and it is not surprising that these cells were confused by investigators who worked without the aid of this method, with either altered lymphocytes (Neisser;[2] Marchoux[3]) or with modified fibroblasts (Baumgarten;[4] Philippson[5]).

The origin of the lepra cell is therefore similar to the origin of the analogous epithelioid cells in tuberculosis. But there is this difference, that the lepra cell is a much better defined group as far as its origin is concerned than the epithelioid cell. The latter in the early stages of the formation of the tubercle is derived from the histiocytes, at least in certain tissues where the vitally stained cells are normally found in considerable numbers; for example, in the liver (Oppenheimer[6]), in the spleen (Goldmann[7]), in lymph glands (Joest and Emshoff[11]), and in the bone marrow (Oliver[12]). But in other tissues, such as alveolar connective tissue, where there are only moderate numbers of histiocytes, it seems certain that there is an added source of epithelioid cells in the fibroblasts. Recent work with methods of tissue culture is also indicating the possibility of their derivation from cells of the circulating blood (Lewis;[13] Maximow[14]). In other words, whereas the epithelioid cells of tuberculosis seem to be a heterogenous group derived from several sources, the lepra cells comprise a much better defined class of cell with a single origin.

CONCLUSION.

1. By means of the method of vital staining it is found that the lepra cell in rat leprosy is derived from the histiocyte.

2. From the similarity in morphology and function of the lepra cells in this condition and those of human leprosy, it seems likely that derivation of the cells in the two conditions is similar.

[11] Joest, E., and Emshoff, E., *Virchows Arch. path. Anat.*, 1912, ccx, 188.
[12] Oliver, J., *J. Exp. Med.*, 1920, xxxii, 153.
[13] Lewis, M. R., *Am. J. Path.*, 1925, i, 91.
[14] Maximow, A., *J. Infect. Dis.*, 1924, xxxiv, 549.

EXPLANATION OF PLATE 4.

FIG. 1. Vitally stained subcutaneous tissues of a leprous rat counterstained with carmine. At the left and bottom of the field are seen nodules composed of light staining lepra cells. Clustering around them and scattered throughout the surrounding granulation tissue are great numbers of dark vitally stained histiocytes. Bausch and Lomb, oc. 1, obj. 1/6.

FIG. 2. Oil immersion field showing the small nodule which lies near the blood vessel in the center of Fig. 1. Section stained with carbolfuchsin and decolorized in acid alcohol. No counterstain was used so that the only stained elements are the bacilli and the granules of vital dye, the nuclei of all cells remaining unstained. The nodule is composed of lepra cells crowded with acid-fast bacilli. At its periphery are seen the vitally stained histiocytes. Some are filled with dye granules, while others contain bacilli and show a marked decrease in dye granules. The transition between vitally stained histiocyte and the bacillus-containing lepra cell is easily seen. Bausch and Lomb, oc. 1, obj. 1/12.

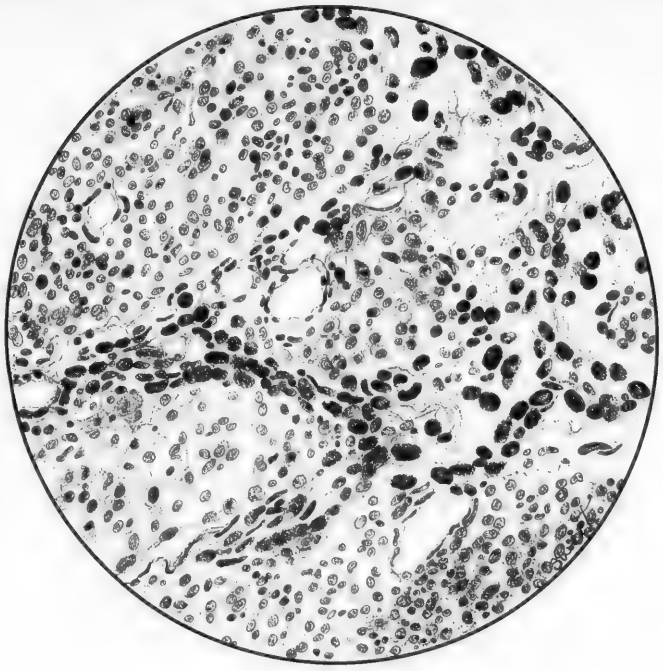

FIG. 1.

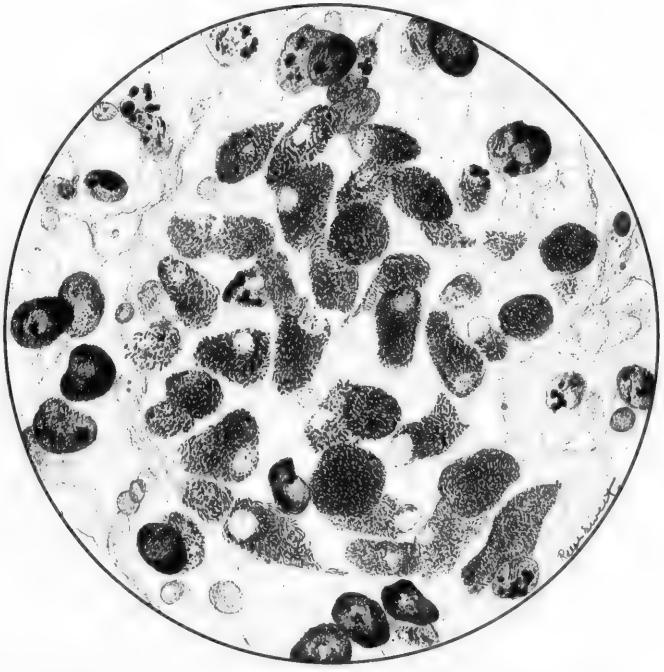

FIG. 2.

(Oliver: Origin of lepra cell.)

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EFFECTS OF SPONTANEOUS DISEASE ON ORGAN WEIGHTS OF RABBITS.

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In recent papers (1, 2) we reported the results of a study of organ weights in apparently normal rabbits considering, first, a group of 350 animals without reference to any factor that might have affected the results obtained. The second paper dealt with possible effects of obscure lesions on the weights of different organs. It was found that the values obtained differed slightly according to the extent of the lesions present, but in all cases they were within the limits of error for any one of the four groups into which the entire series was divided. It thus appeared that the presence of disease processes produced no marked alteration in the mass or mass relations of organs that were not directly affected by the disease, so long as the animal remained in apparent good health; that is, free from signs and symptoms of disease. It was pointed out, however, that even the small differences noted suggested the occurrence of progressive changes in organ weight which became more and more apparent as the disease progressed to the point of clinical recognition.

A study of organ weights in rabbits presenting obvious manifestations of disease of spontaneous origin was carried out parallel with the study of apparently normal rabbits for the purpose of determining whether any relation could be detected between physical constitution, as represented by organ weight, and functional activity, as measured by the efficiency of the reaction to disease. The object of the present paper is to report the results of this investigation without reference to any effects that might be attributable to the character of the disease or the nature of the lesions. These aspects of the problem will be reserved for future consideration.

Methods and Material.

The results to be reported in this paper are based on a study of 127 male rabbits comparable in all respects to those used in the study of normal rabbits except for the presence of physical signs or symptoms of disease of spontaneous origin. Most of the animals were originally selected for the normal series or for some other experiment but were not used for such purposes on account of the development of clinical manifestations of disease while under observation; other animals were selected from the general stock to supplement the group obtained in this way.

The diseases presented by animals of this group were, for the most part, chronic affections such as are apt to develop among laboratory stocks. They included chiefly infections of the nasal passages and sinuses (snuffles) together with infections of the middle ear and mastoids, subcutaneous and deep seated abscesses, coccidiosis, nephritis, and bronchopneumonia. An examination was also made of a small group of animals that died or were killed in the terminal stages of such acute diseases as hemorrhagic septicemia, bronchopneumonia, and enterocolitis. The criteria for inclusion of an animal in this series were the presence of such signs or symptoms of disease as a mucopurulent or purulent discharge from the nose, incoordination or ataxia, inflammatory swellings or accumulations of pus in the tissues, persistent loss of weight or weakness and emaciation, and profuse and persistent diarrhea, or death from unsuspected or obscure disease applicable, in particular, to acute infections.

The methods of conducting the investigation were the same as those employed in the case of normal rabbits, including observation for considerable periods of time as a means of making an appropriate clinical classification.

The classification of animals that was used in this study was chosen with reference to the efficiency of the reaction to disease. A primary division of progressive, and non-progressive or regressive conditions was made and each of these was further divided, giving the following groups arranged in the order of decreasing efficiency.

1. Non-progressive affections.
   Effectual reaction.
   - A. Chronic disease with definite or well advanced improvement or complete recovery.
   - B. Chronic diseases with stationary or uncertain course.

2. Progressive affections.
   Ineffectual reaction.
   - C. Chronic progressive diseases.
   - D. Acute infections with fatal termination.

This classification assumes that the course of disease is towards death or recovery and that the termination and rate of progress in either direction bear some general relation to the efficiency of the reaction, hence, animals dying acutely would be regarded as displaying the least efficient and those that recover, the most efficient reaction. The grouping of material for analysis and the presentation of results are

based on this general conception. These four clinical divisions form the basis for the grouping of data in Table I under corresponding group designations.

The results are recorded in two forms. The numerical values for organ weights are given in Table I with a normal value for each set of figures to facilitate comparison. In Text-figs. 1 to 7, an effort is made to correlate the results obtained with those obtained for normal rabbits on the basis of mean organ weights and coefficients of variation. For this purpose, two groups of animals are used, one representing the apparently normal and the other the obviously diseased animals. The normal animals are divided into four groups, as in the second paper of this series (2), according to the presence or absence and the extent of the lesions found at autopsy. Group 1 is composed of rabbits that showed no gross lesions, Group 2, of animals with slight lesions, and Groups 3 and 4, of animals with moderate and marked lesions respectively. The diseased animals are subdivided into two classes: those showing relatively high resistance or efficient reactions, and those showing relatively low resistance or ineffectual reactions. The subdivisions of these two groups are explained above.

In this way, we have been able to arrange the values for normal and for diseased rabbits in the form of a progressive series, using postmortem findings in one group of animals as the connecting link with the other. Beginning at the middle of each line and proceeding to either the right or the left, we have, first, the value for the total number of normal rabbits, second, the value for those that showed no lesions at autopsy, etc., with the scale of abnormality increasing or the scale of efficiency diminishing until we reach the groups in which disease was apparent during life. In the case of animals of relatively low resistance (right) the terminal event is death, while in those of higher resistance (left) it is recovery, so that the downward progression in the scale of efficiency is continuous in one case but is ultimately reversed in the other with what may be regarded as a change toward normality.

In the graphic representation of results, mean organ weights are compared on the basis of a percentage deviation from the normal or the value obtained for the 350 normal rabbits, while, in the case of coefficients of variation, the actual values are recorded. In one set of figures (Text-figs. 1 to 4) the organs are grouped and compared first on the basis of mean weights, including both the actual and relative weights, then on the basis of coefficients of variation. In the other figures (Text-figs. 5 to 7), the organs are taken separately in order to bring out the relation of all values for each organ.

The lymph nodes have been omitted from these figures on the basis of insufficient data due to the fact that many of these animals were autopsied prior to the inclusion of lymph nodes in our regular scheme of investigations.

No available data have been excluded in making any of the calculations. In Table I, two values are given for thyroid weights of animals in Group A with a preference indicated for a value obtained by the omission of a weight for one animal which presented a definite goiterous enlargement of the thyroid. The figures given in brackets include this weight. A similar discrimination is to be found in the

TABLE I.

Results Obtained for Body and Organ Weights According to Groups.

Organ.	Group.	No. of animals.	Arithmetical mean.	Median.	Minimum.	Maximum.	Standard deviation.	Probable error.	Coefficient of variation.
			gm.	*gm.*	*gm.*	*gm.*	± *gm.*	± *gm.*	*per cent*
Gross body weight.	Normal.	350	2240	2225	1400	3500	353	238	15.7
	A	16	2370	2350	1875	2875	373	252	15.7
	B	53	2385	2275	1325	3475	436	294	18.3
	C	50	2013	2100	1230	2750	345	233	17.1
	D	8	1922	—	1500	2250	236	159	12.3
Gastrointestinal mass.	Normal.	350	416	410	115	710	80.9	54.5	19.4
	A	16	422	410	310	565	74.1	49.9	17.5
	B	53	409	400	290	580	71.1	47.9	17.3
	C	50	376	390	190	595	105.8	71.4	28.1
	D	8	381	—	300	460	49.6	33.4	13.0
Net body weight.	Normal.	350	1849	1800	1150	3010	323	218	17.5
	A	16	1948	1885	1310	2510	313	211	16.0
	B	53	1975	1920	1030	3030	406	274	20.5
	C	50	1637	1675	1015	2305	277	187	16.9
	D	8	1541	—	1153	1850	208	140	13.4
Heart.	Normal.	348	5.26	5.12	3.2	10.18	0.96	0.64	18.2
Actual.	A	16	5.72	5.7	3.74	7.45	0.97	0.65	16.9
	B	53	5.7	5.48	4.0	7.9	1.01	0.678	17.6
	C	50	5.12	5.04	2.7	8.45	1.04	0.7	20.3
	D	6	5.63	—	4.54	7.65	1.05	0.71	18.6

Relative.	Normal.	348	2.85	2.81	1.95	4.42	0.35	0.24	12.3
	A	16	2.95	3.02	2.41	3.35	0.26	0.18	8.8
	B	53	2.92	2.8	2.43	4.48	0.36	0.24	12.3
	C	50	3 15	3.08	2.26	4.95	0.467	0.315	14.8
	D	6	3.84	—	3.19	4.89	0.56	0.38	14.6
Liver. Actual.	Normal.	350	84.3	80.0	50.0	150.0	19.7	13.3	23.4
	A	16	89.2	84.0	60.0	135.0	20.7	13.9	23.2
	B	53	77.4	74.0	43.0	127.0	16.6	11.2	21.4
	C	50	80.8	75.0	30.0	225.0	27.1	18.3	33.5
	D	6	74.8	—	65.0	96.0	11.5	7.8	15.3
Relative.	Normal.	350	46.5	44.9	23.2	97.4	11.84	7.99	25.5
	A	16	46.5	44.6	35.5	84.0	11.67	7.87	25.1
	B	53	40.3	37.3	24.5	78.9	10.4	7.01	25.8
	C	50	49.9	46.9	29.0	157.4	19.01	12.82	38.1
	D	6	51.8	—	37.7	61.9	9.97	6.72	19.2
Kidneys. Actual.	Normal.	348	12.7	12.5	7.15	22.73	2.07	1.4	16.3
	A	16	14.15	13.1	10.04	19.39	3.34	2.25	23.6
	B	53	13.7	13.34	9.63	22.8	2.6	1.75	18.9
	C	50	13.76	12.85	9.17	25.2	3.07	2.07	22.3
	D	6	14.4	—	12.25	21.37	3.16	2.13	21.9
Relative.	Normal.	348	6.97	6.84	3.45	17.28	1.26	0.85	18.1
	A	16	7.22	7.4	5.45	8.53	0.76	0.51	10.5
	B	53	7.02	6.88	4.68	11.65	1.32	0.89	18.8
	C	50	8.68	7.87	5.12	21.17	2.59	1.75	29.8
	D	6	9.9	—	7.37	13.66	2.15	1.45	21.7

TABLE I—*Continued.*

Organ.	Group.	No. of animals.	Arithmetical mean.	Median.	Minimum.	Maximum.	Standard deviation.	Probable error.	Coefficient of variation.
			gm.	*gm.*	*gm.*	*gm.*	± *gm.*	± *gm.*	*per cent*
Spleen.	Normal.	347	0.99	0.9	0.08	3.2	0.436	0.294	44.0
Actual.	A	16	1.013	0.82	0.63	2.45	0.448	0.302	44.2
	B	52	1.099	1.05	0.45	2.7	0.512	0.345	46.6
	C	50	1.089	1.02	0.08	3.2	0.714	0.482	65.5
	D	5	0.808	—	0.42	1.82	0.521	0.351	64.4
Relative.	Normal.	347	0.531	0.49	0.035	1.714	0.214	0.144	40.3
	A	16	0.536	0.47	0.298	1.218	0.23	0.155	42.8
	B	52	0.58	0.536	0.205	1.55	0.277	0.187	47.7
	C	50	0.674	0.655	0.05	1.91	0.334	0.225	49.5
	D	5	0.537	—	0.283	1.055	0.278	0.188	51.7
Thymus.	Normal.	349	2.21	2.13	0.45	5.9	0.848	0.572	38.4
Actual.	A	16	1.764	1.6	0.735	3.45	0.643	0.434	36.4
	B	53	2.092	2.09	0.342	3.75	0.73	0.49	34.9
	C	50	1.391	1.16	0.115	3.7	0.947	0.639	68.1
	D	5	1.798	—	0.712	3.28	0.838	0.565	46.6
Relative.	Normal.	349	1.23	1.147	0.334	2.72	0.451	0.304	36.6
	A	16	0.893	0.881	0.44	1.57	0.249	0.168	27.8
	B	53	1.073	1.078	0.203	1.84	0.365	0.246	34.0
	C	50	0.807	0.647	0.069	2.278	0.546	0.368	67.8
	D	5	1.158	—	0.537	2.09	0.503	0.339	43.4

Testicles.	Normal.	290	4.86	4.85	0.81	9.12	1.63	1.1	35.5
Actual.	A	16	5.41	4.94	1.32	8.05	1.11	0.75	20.5
	B	49	5.2	5.04	2.7	8.56	1.21	0.82	23.2
	C	47	4.45	4.58	0.78	9.08	1.73	1.17	38.8
	D	6	4.73	—	2.53	6.6	1.42	0.96	30.0
Relative.	Normal.	290	2.61	2.62	0.47	4.93	0.7	0.47	26.9
	A	16	2.74	2.73	1.0	4.03	0.77	0.52	28.1
	B	49	2.7	2.59	1.1	4.43	0.6	0.41	22.3
	C	47	2.71	2.72	0.5	4.58	0.87	0.59	32.1
	D	6	3.17	—	2.19	4.41	0.73	0.49	23.0
Brain.	Normal.	150	9.31	9.24	7.42	12.03	0.78	0.53	8.4
Actual.	A	16	9.17	8.9	8.05	11.6	0.87	0.59	9.4
	B	45	9.22	9.15	7.05	11.0	0.75	0.51	8.1
	C	40	9.25	9.39	7.56	11.84	0.91	0.61	9.7
	D	6	9.17	—	8.25	11.39	1.03	0.69	11.2
Relative.	Normal.	150	5.11	5.06	3.33	8.16	0.87	0.58	16.9
	A	16	4.82	4.49	3.6	6.45	0.71	0.48	14.7
	B	45	4.75	4.7	3.44	6.28	0.69	0.47	14.5
	C	40	5.68	5.43	4.37	9.27	1.01	0.68	17.7
	D	6	6.33	—	5.14	7.85	0.97	0.65	15.3

TABLE I—*Continued.*

Organ.	Group.	No. of animals.	Arithmetical mean.	Median.	Minimum.	Maximum.	Standard deviation.	Probable error.	Coefficient of variation.
			gm.	*gm.*	*gm.*	*gm.*	± *gm.*	± *gm.*	*per cent*
Thyroid	Normal.	349	0.231	0.185	0.085	1.57	0.162	0.109	70.3
Actual.	A	16	0.252	0.21	0.115	0.55	0.121	0.082	48.0
			(0.37)	(0.214)	(0.115)	(2.14)	(0.472)	(0.318)	(127.5)
	B	53	0.294	0.215	0.13	1.375	0.232	0.157	79.0
	C	50	0.214	0.18	0.115	0.53	0.097	0.066	45.4
	D	8	0.204	—	0.11	0.302	0.062	0.041	30.0
Relative.	Normal.	349	0.123	0.098	0.05	0.73	0.076	0.051	61.5
	A	16	0.131	0.114	0.071	0.315	0.062	0.042	47.2
			(0.176)	(0.117)	(0.071)	(0.851)	(0.179)	(0.121)	(101.4)
	B	53	0.148	0.119	0.063	0.658	0.101	0.068	67.8
	C	50	0.129	0.117	0.067	0.312	0.051	0.034	39.9
	D	8	0.131	—	0.095	0.181	0.031	0.021	23.6
Parathyroids.	Normal.	349	0.01256	0.012	0.002	0.035	0.00486	0.00328	38.7
Actual.	A	16	0.0156	0.013	0.007	0.03	0.00623	0.0042	40.0
	B	53	0.0156	0.015	0.005	0.062	0.00814	0.00549	52.2
	C	50	0.0141	0.013	0.004	0.032	0.00584	0.00394	40.0
	D	8	0.0179	—	0.01	0.028	0.0065	0.00438	36.3
Relative.	Normal.	349	0.00692	0.00631	0.00109	0.02175	0.00278	0.00187	40.1
	A	16	0.00824	0.00774	0.00401	0.01363	0.00325	0.00219	39.4
	B	53	0.0078	0.0076	0.00301	0.02046	0.00263	0.00177	33.7
	C	50	0.00876	0.00787	0.00358	0.02415	0.00385	0.0026	43.9
	D	8	0.0128	—	0.0065	0.01695	0.00365	0.00246	28.4
Hypophysis.	Normal.	348	0.028	0.028	0.015	0.044	0.00517	0.00349	18.5
Actual.	A	16	0.0304	0.03	0.022	0.043	0.0051	0.00344	16.7
	B	53	0.0305	0.03	0.02	0.05	0.0066	0.00445	21.6
	C	50	0.02936	0.028	0.015	0.057	0.0073	0.0049	24.8
	D	8	0.0284	—	0.015	0.04	0.0078	0.00526	27.4

Relative.	Normal.	348	0.0154	0.0153	0.00728	0.0294	0.0031	0.00209	20.3
	A	16	0.01588	0.01567	0.01122	0.02095	0.00255	0.00172	16.1
	B	53	0.0158	0.0156	0.0075	0.0279	0.0036	0.00242	22.8
	C	50	0.01812	0.0168	0.0087	0.03304	0.0047	0.0032	25.9
	D	8	0.0185	—	0.0113	0.026	0.0047	0.0032	25.4
Suprarenals.	Normal.	349	0.3801	0.358	0.115	1.05	0.155	0.1046	40.8
Actual.	A	16	0.4514	0.42	0.22	0.79	0.139	0.0938	30.8
	B	53	0.458	0.392	0.15	0.92	0.1927	0.1299	42.1
	C	50	0.4705	0.4	0.157	1.3	0.2442	0.1647	51.8
	D	8	0.6299	—	0.3	1.539	0.3808	0.2568	60.4
Relative.	Normal.	349	0.2088	0.1967	0.0804	0.53	0.075	0.0506	35.9
	A	16	0.2357	0.2156	0.115	0.453	0.0742	0.05	31.4
	B	53	0.231	0.2099	0.0882	0.464	0.0855	0.0577	37.0
	C	50	0.2836	0.262	0.1245	0.613	0.1276	0.0861	45.0
	D	8	0.4008	0.1739	0.1739	0.832	0.2022	0.1363	50.4
Pineal gland.	Normal.	348	0.01609	0.015	0.005	0.03	0.00429	0.00289	26.7
Actual.	A	16	0.0178	0.017	0.012	0.025	0.00309	0.00208	17.3
	B	53	0.0175	0.0155	0.01	0.034	0.0056	0.00378	32.0
	C	49	0.01586	0.015	0.009	0.03	0.00425	0.00287	26.8
	D	7	0.0167	—	0.005	0.04	0.01044	0.00704	62.5
Relative.	Normal.	348	0.00885	0.00868	0.00273	0.01873	0.00245	0.00165	27.7
	A	16	0.00946	0.00974	0.00553	0.01527	0.00223	0.0015	23.5
	B	53	0.00901	0.00829	0.00501	0.0234	0.00308	0.00208	34.2
	C	49	0.00995	0.00962	0.00528	0.01847	0.00293	0.00198	29.4
	D	7	0.01113	—	0.0029	0.02402	0.0061	0.00411	54.8

TABLE I—*Concluded.*

Organ.	Group.	No. of animals.	Arithmetical mean.	Median.	Minimum.	Maximum.	Standard deviation.	Probable error.	Coefficient of variation.
			gm.	*gm.*	*gm.*	*gm.*	± *gm.*	± *gm.*	*per cent*
Popliteal lymph nodes. Actual.	Normal.	242	0.2554	0.245	0.085	0.72	0.0824	0.0556	32.3
	A	9	0.2216	0.22	0.13	0.315	0.0624	0.0421	28.1
	B	43	0.2287	0.21	0.115	0.375	0.0656	0.0442	28.7
	C	30	0.2278	0.21	0.075	0.51	0.097	0.0654	42.5
	D	—	—	—	—	—	—	—	—
Relative.	Normal.	242	0.1357	0.1361	0.0525	0.382	0.0487	0.0329	35.9
	A	9	0.111	0.1147	0.0518	0.1719	0.0389	0.0262	35.0
	B	43	0.118	0.11	0.0636	0.2039	0.0344	0.0232	29.1
	C	30	0.141	0.1355	0.0522	0.304	0.0484	0.0327	34.3
	D	—	—	—	—	—	—	—	—
Axillary lymph nodes. Actual.	Normal.	242	0.1759	0.175	0.04	0.4	0.0598	0.0403	34.0
	A	9	0.1516	0.13	0.075	0.23	0.0514	0.0347	33.9
	B	43	0.1482	0.145	0.05	0.41	0.0557	0.0376	37.6
	C	30	0.1284	0.118	0.035	0.275	0.0503	0.0339	39.1
	D	—	—	—	—	—	—	—	—
Relative.	Normal.	242	0.0972	0.091	0.0187	0.242	0.0378	0.0255	38.9
	A	9	0.0753	0.084	0.0299	0.125	0.0279	0.0188	37.1
	B	43	0.0801	0.0751	0.0332	0.282	0.0426	0.0287	51.9
	C	30	0.0786	0.0704	0.0206	0.1652	0.0319	0.0215	40.6
	D	—	—	—	—	—	—	—	—

Deep cervical lymph nodes.	Normal.	124	0.1558	0.14	0.035	0.405	0.0752	0.0494	47.0
Actual.	A	4	0.183	—	0.1	0.31	0.0861	0.0544	47.0
	B	24	0.2583	0.22	0.095	0.67	0.1422	0.0959	55.1
	C	15	0.289	0.17	0.115	0.84	0.1921	0.1296	66.4
	D	—	—	—	—	—	—	—	—
Relative.	Normal.	124	0.0821	0.0724	0.0205	0.211	0.0371	0.025	45.2
	A	4	0.0893	—	0.0613	0.1302	0.0256	0.0173	28.6
	B	24	0.1327	0.1113	0.0538	0.325	0.0717	0.0484	54.0
	C	15	0.1774	0.1255	0.0801	0.555	0.1141	0.077	64.3
	D	—	—	—	—	—	—	—	—
Mesenteric lymph nodes.	Normal.	133	3.488	3.32	1.34	9.19	1.31	0.88	37.5
Actual.	A	9	3.26	3.27	2.17	4.65	0.68	0.46	20.9
	B	39	2.99	2.9	1.36	4.85	0.827	0.558	27.6
	C	34	2.94	2.9	1.25	5.5	1.01	0.68	34.3
	D	—	—	—	—	—	—	—	—
Relative.	Normal.	133	1.828	1.763	0.72	3.71	0.642	0.43	35.1
	A	9	1.637	1.39	0.912	2.86	0.543	0.366	33.1
	B	39	1.52	1.505	0.714	3.19	0.443	0.299	29.1
	C	34	1.75	1.66	0.821	3.49	0.558	0.376	29.6
	D	—	—	—	—	—	—	—	—

text-figures where the line for the thyroid is continued to the value obtained by omitting this one weight instead of the value with this weight included, as indicated in the figures.

RESULTS.

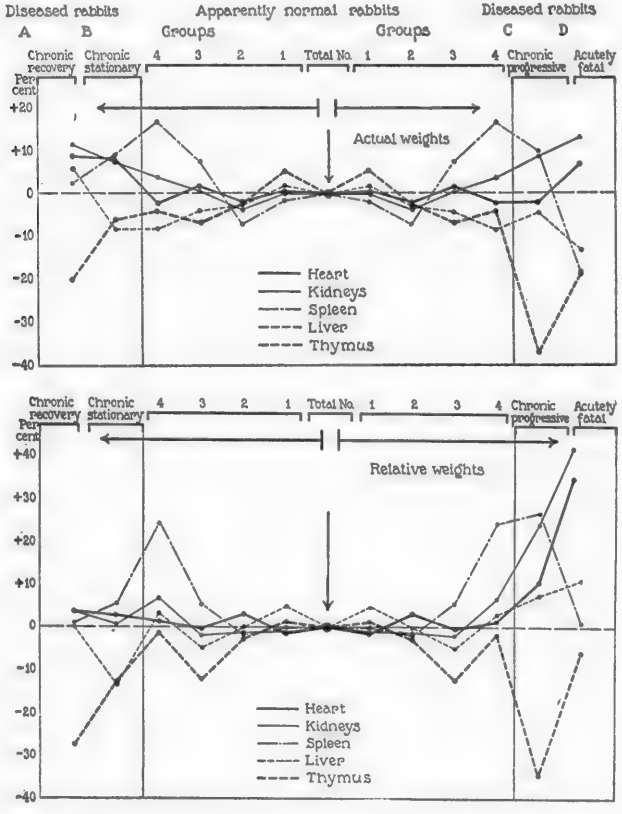
The results of the observations made on organ weights of rabbits presenting clinical manifestations of disease of spontaneous origin are recorded in Table I and Text-figs. 1 to 7.

DISCUSSION AND CONCLUSIONS.

An examination of the figures given in Table I will show that in most instances the values obtained for the four groups of diseased rabbits do not differ greatly from those of the normal controls. Mean values for actual organ weight may be larger or smaller and occasionally the maximum and minimum values exceed the normal limits, but weights per kilo of net body weight show a more consistent behavior; mean weights for the heart, liver, kidneys, spleen, thyroid, parathyroids, suprarenals, hypophysis, pineal gland, and deep cervical lymph nodes tend to run high, while those for the thymus, popliteal, axillary, and mesenteric lymph nodes run low; the brain weight is low in Groups A and B and high in Groups C and D. Similar conditions obtain for the probable error and coefficients of variation. Still, if the analysis of results were limited to a consideration of the actual magnitude of the deviation from the normal, it would be found that nearly all of the weights are within the limits of error for normal rabbits.

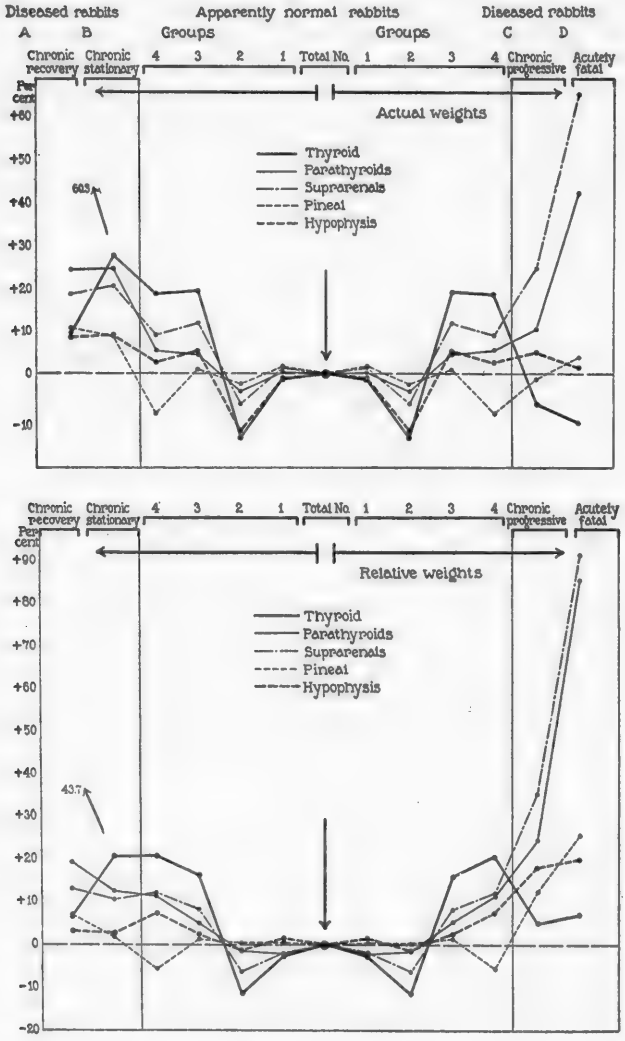
This raises the question of the extent of the deviation from the normal that may be regarded as significant when normal standards are fixed with due regard for circumstances that may affect normal values. The standards employed in the present instance were worked out with a view to including all variations in weight that are likely to occur in normal rabbits. They should include, therefore, most functional variations, and from the results recorded in this and the previous paper, together with a large volume of unpublished data, it seems that adequate standards of normality may indeed include all variations in organ weight that are of functional origin and hence all variations that are likely to occur in consequence of disease, except

those of organs that are themselves the seat of some disease process or are directly affected by the disease. Under these circumstances, reliance on differences in weight with corrections for probable error



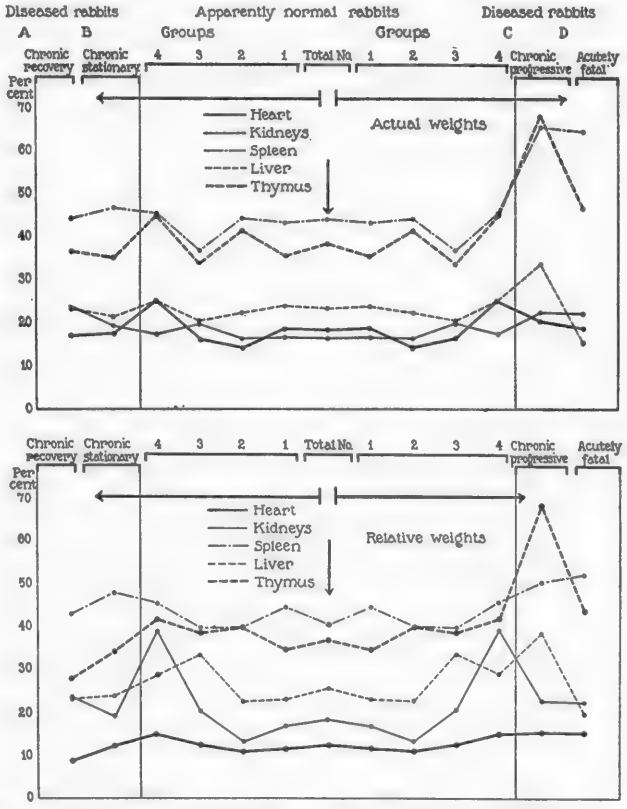
TEXT-FIG. 1. Percentage deviation of actual and relative weights of organs from the mean value obtained for apparently normal rabbits. The values for different groups of animals are arranged according to the extent of lesions found at autopsy or the clinical course of disease.

would tend to disclose only those changes in organ weight that are direct consequences of disease and to obscure those that are due to altered functional activity. Functional alterations are the ones in



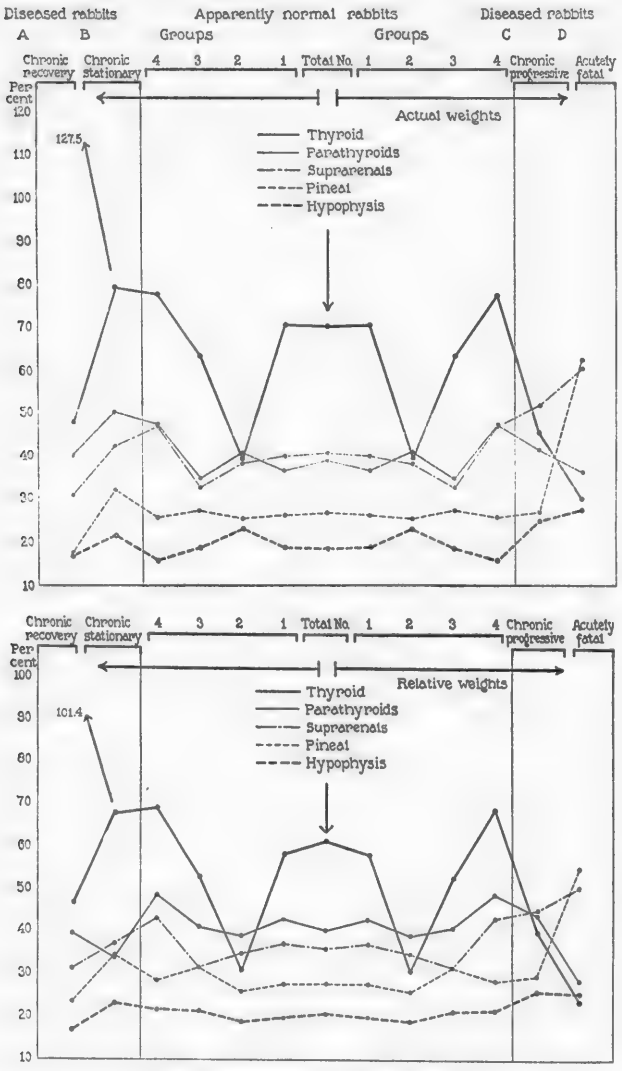
TEXT-FIG. 2. Same as Text-fig. 1.

which we are chiefly interested, and the fact that the changes found are within the limits of normal variation is important evidence of their nature.



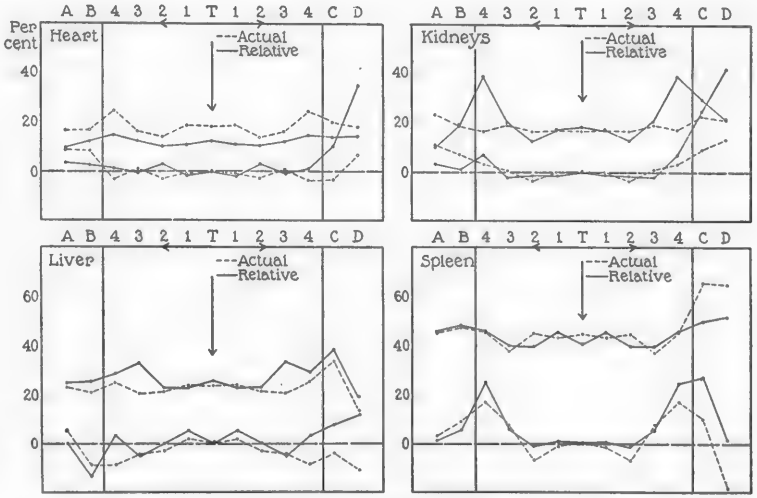
TEXT-FIG. 3. Coefficients of variation for actual and relative weights of organs of apparently normal and diseased rabbits.

If we consider not only the magnitude of the variation but the direction and order of arrangement of values as given, it will be found that there is an indication of a relation between the deviation from the normal and the clinical status of the several groups of animals with respect to the efficiency of the reaction to disease. This is



TEXT-FIG. 4. Same as Text-fig. 3.

especially true of Groups A, B, and C which represent animals with chronic affections. As a rule, mean weights per kilo of net body weight for Groups A and B are nearer the normal than those for Group C, and the coefficients are either nearer the normal or smaller. The results for Group D are more irregular; in a few instances they show the closest approximation to the normal or the smallest coefficient of variation but in most cases the relation is reversed. This irregular-



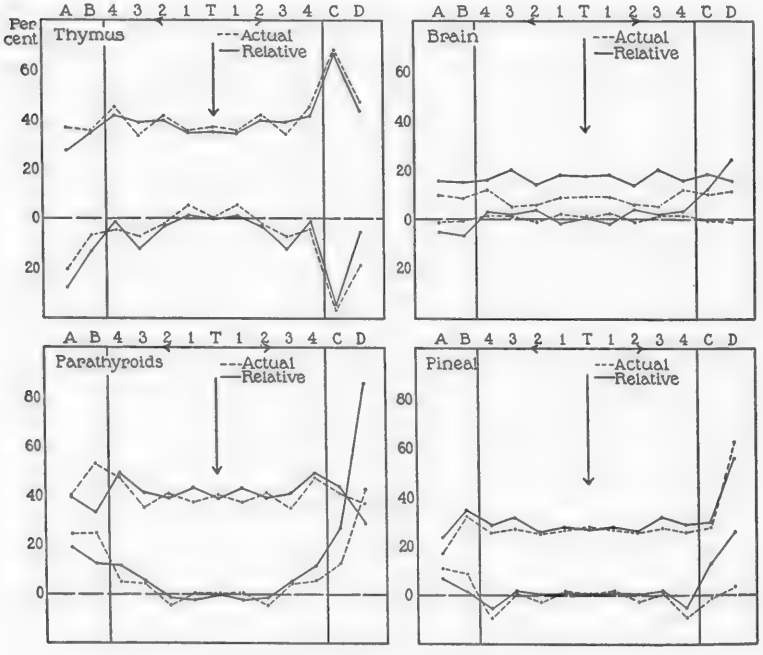
TEXT-FIG. 5. Percentage deviation of weights and coefficients of variation for individual organs as in Text-figs. 1 to 4. The two upper lines represent the values for the coefficients in each case.

ity may be attributable, in part, to the very small number of animals in the group and, in part, to the acute nature of the disease. The group is, however, the most homogeneous of all.

The significance of these findings can be brought out best by correlating the results in animals presenting clinical manifestations of disease with those obtained in apparently normal rabbits, grouped according to the lesions found at autopsy. The results of comparisons of this kind are shown in Text-figs. 1 to 7. In the first series of figures (Text-figs. 1 to 4), organs are grouped and functions are

considered separately, while in the second (Text-figs. 5 to 7), results for individual organs are combined. In analyzing the relationships shown by individual groups of animals, greater significance is to be attached to the form of the curves than to the actual values recorded.

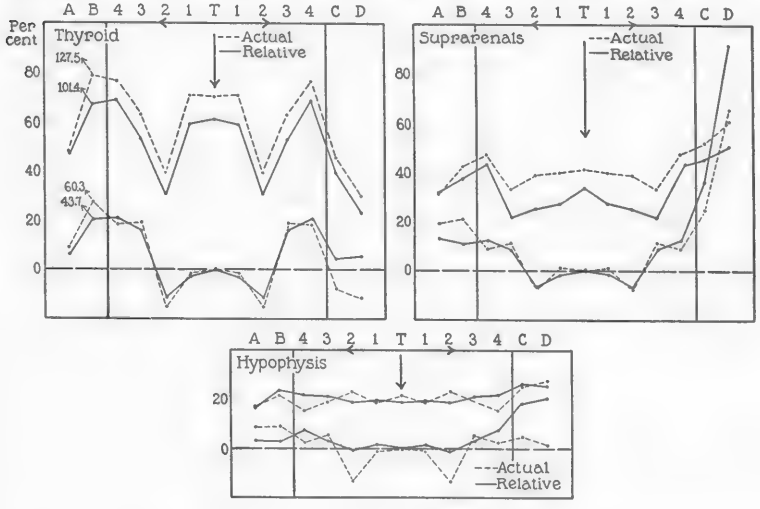
If we consider first the general relationships, as indicated by mean weights (Text-figs. 1 and 2), it will be seen that as we pass from a



TEXT-FIG. 6. Same as Text-fig. 5.

theoretical normal value through various degrees of abnormality, some organs change very little or not at all while others show decided variations. At first, the lines are closely grouped and they tend to follow a common direction, whether upward or downward, but, as they continue outward in either direction, they diverge or become scattered so that the relation to the normal or of one organ to another differs according to the extent of the lesions present or the clinical

course of disease. There is, however, a distinct difference between the conditions shown for animals in which the disease was stationary or tending toward recovery and those in which the disease was progressing toward a fatal termination. In the first case, the values change very little or, in the end, tend to assume a position nearer the normal. In animals of relatively low resistance, nearly all organs show a continuation of the lines upward or downward, as the case may be, which is distinctly greater than that shown on the opposite side of the chart.



TEXT-FIG. 7. Same as Text-fig. 5.

Moreover, the general effect is to increase the dispersion of values and the extent of the divergence from normal. This is especially applicable to chronic progressive affections and does not apply with equal force to acutely fatal conditions which may not last sufficiently long to permit of such changes.

There are striking exceptions to this general rule. For example, the thymus shows a reduction in weight for every departure from the normal and no evidence of a return toward normal with the initiation of recovery; this doubtless would come as a later development. Again, there is a decrease instead of an increase in the weight of the thyroid in animals with chronic progressive and acutely fatal diseases as

compared with the maintenance of a high weight level in animals that displayed a more effectual resistance. In the light of other evidence, this feature of the thyroid curve appears to be characteristic of the difference between an effectually sustained reaction and an ineffectual or failing reaction.

Coefficients of variation show a similar situation with respect to the constancy of weights in different groups of animals. These values (Text-figs. 3 and 4) are compared on a different basis from mean organ weight with a view to preserving the indication which they afford of the tendency of different organs to vary as well as the tendency of a given organ to vary under different circumstances. This greatly reduces the scale of the variations recorded so that the magnitude of the variation shown cannot be compared directly with that of the change in organ weight. There is, however, a similarity in the form of the two sets of curves, and any decided change in one value is nearly always accompanied by a decided change in the other; these may take the same or opposite directions as shown in Text-figs. 5 to 7.

Without attempting to analyze these changes in detail, it may be said that, in general, the coefficients of variation tend to be low, within the limits of normal, until the borderline groups are reached. At this point, the coefficient is usually increased. In animals that display an effectual reaction (Groups A and B), there is either no further increase or the value diminishes, while in the case of chronic progressive and acutely fatal affections (Groups C and D), the coefficient may remain high, increase, or diminish but, as a rule, the coefficient is larger or the change greater than the corresponding values for Groups A and B; that is, among animals that display an effectual reaction, there is evidence of the preservation of greater constancy with respect to organ weight than among those that display an ineffectual reaction.

The conditions presented by individual organs require no detailed discussion; the form and the magnitude of the change in weight and coefficients of variation as recorded for the several groups of animals are clearly shown in Text-figs. 5 to 7.

This analysis of results shows that the physical constitution of rabbits presenting clinical manifestations of disease differs in several

essential respects from that of normal rabbits. Any departure from the normal appears to be accompanied by some change in organ weight and in the coefficients of variation for organ weight which proceeds parallel with pathological or clinical evidence of disease. The direction of the change varies with different organs but in most instances the first movement is in the direction of a reduction in weight with a smaller coefficient of variation; this change occurs sooner in the glands of internal secretion than in the larger parenchymatous organs (Text-figs. 1 to 4); with increasing abnormality weights and coefficients tend to increase, reaching a maximum in animals that present clinical as well as pathological evidence of disease. At this point the direction of the change varies; if the disease progresses toward a fatal termination most organs show a continued increase in weight while others show an abrupt reduction with irregular changes in the coefficients of variation, but, if the course of events is toward recovery, the organ weights and coefficients tend to diminish or return to normal.

The finding of some difference between the weights of organs of normal and diseased animals is not surprising, as such differences might be caused by a disturbance of nutrition or by retrograde changes in the substance of the organ as direct or indirect consequences of disease. It is not unlikely that these factors, and perhaps others, played some part in the production of the changes found, and if our observations had been limited to animals with outspoken manifestations of disease, it would have been difficult or impossible to determine whether the differences noted represented anything more than results of the action of factors of this kind. With the material available, however, it is apparent that these were not the sole causes.

We have for comparison a group of apparently normal and a group of obviously diseased rabbits. The normal rabbits were in excellent physical condition but many of them (20 per cent) showed lesions at autopsy that were comparable to the majority of those found among the diseased animals. The outstanding difference between the two groups was, therefore, the physical condition or state of nutrition and the presence or absence of other clinical signs and symptoms of disease. Among the diseased animals there were also differences. Most of them were well nourished and, while the proportion of thin or emaciated animals in Group C was larger than that of the other groups,

all groups contained a few such animals; among the animals with chronic diseases, the lesions were also comparable, so that the chief difference was again not one of nutrition or of extent of lesions but the capacity for the control of the disease as indicated by the progress toward death or recovery.

When the results for normal and diseased animals are arranged in serial order, and analyzed with reference to the conditions presented by successive groups, it is found that the changes in organ weight and coefficients of variation do not conform to any scheme of nutritional gradations or even to the extent of lesions; they tend, however, to be consecutive and to follow the course of disease, showing a departure from the normal which, in general, is inversely proportional to the efficiency of the reaction displayed by the several groups of animals. Moreover, the changes that occur are in most cases within the limits of functional variations for normal rabbits.

It appears to be reasonably certain, therefore, that the changes in organ weight and coefficients of variation are of the order of functional reactions which are modified to some extent by other factors.

SUMMARY.

A group of 127 rabbits presenting clinical manifestations of disease of spontaneous origin was studied with a view to determining whether any relation could be detected between physical constitution as represented by organ weights and functional activity as measured by the efficiency of the reaction to disease. The results of the investigation are presented in tabular form and the values obtained are compared, by means of graphs, with corresponding values for normal rabbits.

It was found that animals that were the subjects of disease showed decided changes in the weights of nearly all organs, changes which appeared to be of functional origin in that the values obtained for organ weights and coefficients of variation tended to conform, in general, with the efficiency of the reaction displayed by the various groups of animals into which the entire series was divided.

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ALLERGIC IRRITABILITY.

III. The Influence of Chronic Infections and of Trypan Blue on the Formation of Specific Antibodies.

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The purpose of this paper is to present observations in confirmation and extension of our previous papers (1, 2) on this general subject, particularly the first paper. We there showed that in the tuberculous guinea pig the production of anti-sheep hemolytic amboceptor was exaggerated. We have now found that the production of agglutinins for *Bacillus typhosus* is likewise increased by preexisting active tuberculosis. We have studied other mild infections, the influence of the dead tubercle bacillus, and trypan blue, finding that hemolytic amboceptor production may be increased by these means. We likewise have been able to increase measurably the production of hemolytic amboceptor by the rabbit through the establishment of an infection with *Bacillus tuberculosis*. These additional observations seem to warrant the conclusion that the phenomenon dealt with is a very general one, as to the species in which it can be made evident, the antigens whose activity may be influenced, and the means which may be effective in altering the animal's reaction capacity. These additional observations and others which will appear incidentally in the body of the paper suggest a further definition of the conception of allergic irritability as we have sought to develop it.

As an incident to our previous observations on increased antibody production we determined the curve of antibody production in the guinea pig and found that it departed from the usual type. The present paper contains some additional information on this point.

LITERATURE.

When preparing our previous paper we overlooked a paper by Clark, Zellmer, and Stone (3). These authors found that the production of agglutinins for *B. typhosus* was increased by the previous administration of heat-killed Gram-positive cocci. Rabbits were used. Some evidence that the rabbits were measurably protected against killing doses of *B. typhosus* was also obtained by these authors. These experiments are of importance as a possible indication of some connection between the experiments on antibody production and the classical experiments of Pfeiffer and Issaeff (4) on immunity induced by the injection of bouillon and other indifferent substances into the peritoneal cavity. It has usually been assumed that the effect obtained by Pfeiffer and Issaeff was of short duration and purely a local manifestation. It may possibly be related in some way to a more general and lasting reaction.

Khanolkar (5) obtained a moderate increase in the production of agglutinins for *B. paratyphosus*, Gärtner, by previous treatment with killed *B. pyocyaneus*. He failed to obtain such an effect with staphylococci and ricin.

Schroeder (6) has observed greatly increased anti-sheep amboceptor production in rabbits which developed abscesses at the site of inoculation of erythrocytes. In control experiments she was successful in showing the similar influence of pneumococcus infection. With staphylococci and *B. pyogenes* she did not succeed in establishing chronic infections and was unable to show any stimulating influence.

Hektoen and Corper (7) have recently reported that they failed to obtain increased antibody production in rabbits under the influence of preexisting tuberculosis infection with any degree of consistency. Their results were not entirely negative, however, even as reflected in their statement of conclusions, and we feel that they are to be interpreted as in essential harmony with our own. This matter will be taken up in greater detail in our discussion.

Gay and Clark (8) found that administration of trypan blue interfered with the production of antibodies. This is interpreted as probably signifying that those cells in the body which have a marked affinity for trypan blue are concerned in a vital way in the production of antibodies, and that this function is interfered with when they are filled with dye. The authors' context implies that the "blocking" experiments of others have sometimes given increased antibody production rather than the anticipated decrease. There is probably no fundamental contradiction between the opposing results, each tending to assign to the reticulo-endothelial cell system a definite place in antibody production.

EXPERIMENTAL.

In a first series guinea pigs belonging to our inbred Family 13 were used. They were given at one time 5 cc. subcutaneously and 5 cc. intraperitoneally of a 20 per cent suspension of washed sheep red blood corpuscles. They were further treated as follows: A group of five received 5 cc. of 1 per cent trypan blue intraperitoneally the day before the sheep blood, and 2 cc. each day thereafter until

the end of the experiment. A second group of five received 0.8 cc. of a 24 hour culture of a streptococcus subcutaneously 2 days before the injection of sheep cells.

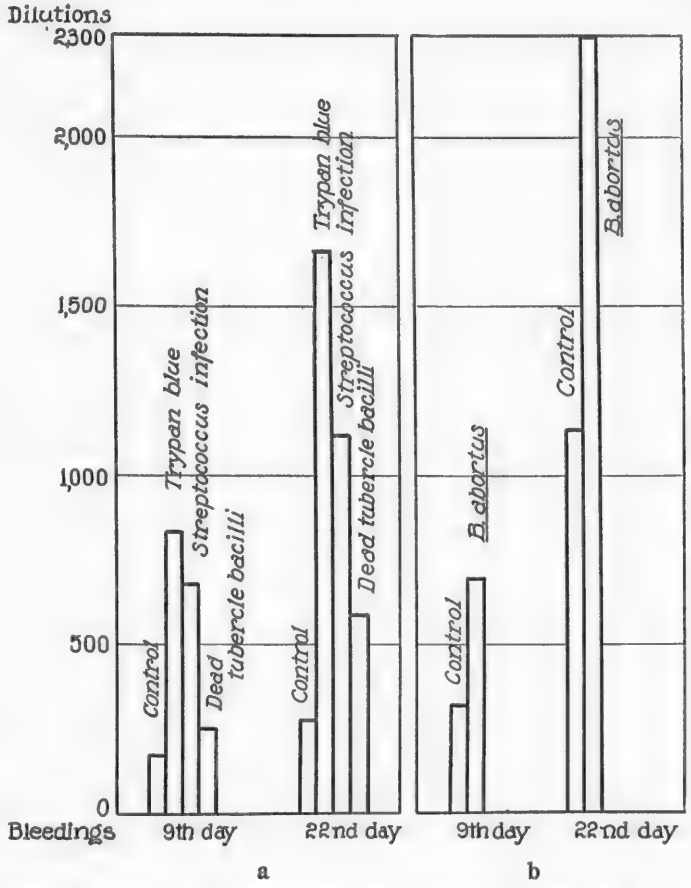

CHART 1, *a* and *b*. (*a*) Average hemolytic amboceptor production of groups of guinea pigs treated with trypan blue, a streptococcus, and dead tubercle bacilli contrasted with a control group. Bleedings on the 9th and 22nd days after the administration of sheep erythrocytes. (*b*) Same for a group infected with *B. abortus* contrasted with a control group.

The streptococcus was one isolated some months earlier from a swollen lymph node in a guinea pig dead of some cause unknown. A third group of five received 2 mg.

of culture of *B. tuberculosis*, Bovine xiv, intraperitoneally 2 days before the sheep cells. The culture was killed by heating to 60°C. for 1 hour. A fourth group of five was used for control, receiving no treatment other than the sheep red cells.

The animals were bled on the 9th and 22nd days following the erythrocyte injections. The sera were tested individually for the minimum hemolytic dose against 0.1 cc. of a 10 per cent suspension of sheep erythrocytes, in the presence of an excess of complement and in a total volume of 1.1 cc. The temperature of the test was 35–36°C., the time 1 hour, and the readings were made at once. For purposes of interpretation the results were averaged for each group. As a check the sera for each group were pooled in equal portions and the combined serum tested at a later time in the same way. The results were in essential accord with the averages of the individual determinations.

The results of this series of tests are shown in Chart 1, *a*.

In a second series ten animals of inbred Family 13 and ten of Family 35 were inoculated with 1 cc. of a suspension of *B. abortus* grown on agar and suspended in normal saline solution to a density of 2.5 on the Gates gauge. 21 days later these animals with five others of each family, selected as controls, were treated with sheep red cells. They were given at one time 5 cc. intraperitoneally and 5 cc. subcutaneously as in the earlier series. These animals were likewise bled on the 9th and 22nd days after the blood injection and the hemolytic titer tested as before.

The results of this series are shown in Chart 1, *b*.

Two experiments were next carried out to see if infection with *Bacillus tuberculosis* would affect the production of antibodies other than sheep cell hemolysin. In the first experiment the first treatment with *Bacillus typhosus* proved insufficient for agglutinin production. A second treatment was therefore given. The tuberculous animals gave much more agglutinin than the normals, but since we have wished for a result uncomplicated by repeated injections with the test antigen, we carried out a second series as follows:

Twenty guinea pigs of inbred Family 13 were given an intraperitoneal injection of 1/100 mg. of *B. tuberculosis*, Bovine xiv. 2 weeks later these animals with ten others used as normal controls were given at the one time 1 cc. intraperitoneally and 1 cc. subcutaneously of a suspension of *B. typhosus*, density with Gates gauge 1.8, which had been heated to 55°C. for 1 hour. All the animals were bled on the 7th, 9th, 11th, 15th, 18th, 20th, and 22nd days after the injection of *B. typhosus*. Equal parts of the serum of each animal were pooled by groups for each day, stored in the ice box, and the whole tested with the same suspension of

B. typhosus that had been used for the injection. The agglutination test was done by the macroscopic method. The tubes were incubated for ½ hour at 56°C., placed in the refrigerator overnight, and read the following morning. The last tube showing complete clearing with heavy flocculi on reshaking was taken as the end-point.

The result of the whole experiment is shown in Chart 2.

Having thus found that a stimulus to antibody production could be attained in several ways, and that the resultant increase could be

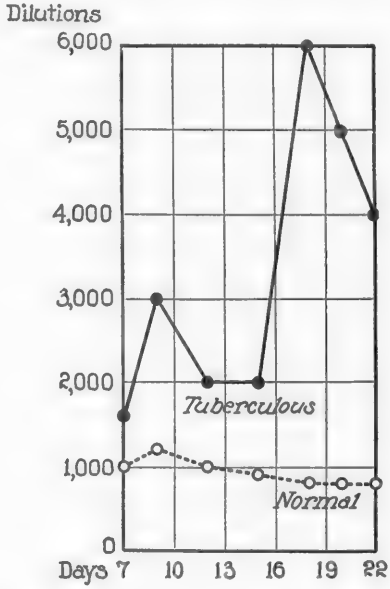

CHART 2. *B. typhosus* agglutinin production in tuberculous and normal animals. The time in days is counted from the administration of *B. typhosus* as described in the text.

observed with a bacterial antibody as well as with an hemolytic amboceptor, we tried to see if the effect could be obtained in an animal other than the guinea pig. We chose to produce anti-sheep hemolytic amboceptor in the rabbit under the modifying influence of an infection with *Bacillus tuberculosis*. The experiment was planned to fulfill conditions as they had been developed in the guinea pig;

that is, it appeared that the infection must be of at least 2 weeks standing if the effect was to be manifest. Three experiments were done.

In the first of these 1/100 mg. of a bovine type culture, No. xviii, was given intraperitoneally. No significant differences in hemolysin production were made out, and when the animals were autopsied it was found that the infection had produced an insignificant amount of disease. In a second experiment 1 mg. of bovine type culture No. xiv was given intravenously. The blood cells were injected on the 16th day. On the 4th, 7th, and 9th days after the erythrocyte injection the amboceptor content of the tuberculous rabbits was lower than in the controls. Thereafter there was but one surviving tuberculous rabbit. As the amboceptor content of the control animals remained stationary over the succeeding 6 days, that in the tuberculous animal increased until on the 16th day it stood at 1/16,000. The average of the controls on this day was 1/5,600 and the maximum among five was 1/9,000.

TABLE I.

Controls.			Tuberculous.		
Rabbit No.	7th day.	18th day.	Rabbit No.	7th day.	18th day.
31	1/2,000	1/1,600	24	1/10,000	Dead.
32	1/4,000	1/5,000	25	1/3,000	"
33	1/5,000	1/3,000	26	1/8,000	"
34	1/4,000	1/1,600	27	1/20,000	1/12,000
35	1/3,000	1/1,600	28	1/8,000	Dead.
			30	1/8,000	1/12,000
Average....	1/3,600	1/2,540		1/9,500	1/12,000

In a third experiment the same culture was used (Bovine xiv). 1/20 mg. was injected intravenously into seven rabbits. 17 days later these together with five controls were given at one time 5 cc. of 20 per cent washed sheep red blood corpuscles intravenously, 5 cc. intraperitoneally, and 5 cc. subcutaneously. They were all bled on the 7th and 18th days succeeding. The results are shown in Table I.

While it is plain that the experiment does not proceed with the same regularity as in the guinea pig, it is nonetheless evident that in the rabbit, under appropriate conditions, an infection with the tubercle bacillus does increase the production of anti-sheep amboceptor. Conditions favorable to the demonstration appear to be the thorough establishment of an infection of considerable, but not of overwhelming severity.

The preceding experiments show that the stimulation of antibody production with which we are concerned is quite a general phenomenon. It can be brought about by a variety of chronic infections; it is exerted against at least two antigens, and is developed in at least two species of animals. Of the means adopted none thus far tested is equal from a quantitative point of view to an active, well established infection with *Bacillus tuberculosis*.

In this connection it has been interesting to compare the effect of repeated injections of red cells in normal and tuberculous animals. The experiment is limited in its scope because of the short length of life of the tuberculous. The results may be simply stated without tabulation. In a group of ten tuberculous animals given three injections of red cells an average amboceptor titration of 1/58,000 was obtained on the 22nd day after the last injection. One of the animals reached 1/100,000. In normal animals the highest individual figure obtained by any number of injections (following the second injection in this instance) was 1/5,500, and the highest average for any group was 1/4,500. As reported in our first paper, a single injection of blood cells in tuberculous animals may give an average titration of over 1/20,000 on the 22nd day, a figure still four or five times the maximum we have been able to attain by repeated blood cell injection in uninfected animals. The stimulus of infection would appear to be more effective in increasing the quantity of antibody produced than the stimulus of several preceding injections of the specific antigen. That the figures for attainable antibody concentration afford the proper basis for comparison of the quantitative effects of the two sorts of stimuli may well be doubted. We have wished to construct complete curves of antibody production for the normal and the tuberculous after repeated blood cell injections. The technical difficulties have not so far been surmounted. It is our impression, however, that for repeated injections, as was shown for single injections in our first paper, the form of the curves would be comparable but at a higher level. It seems likely, in other words, that the factor by which each succeeding injection would increase the results of its predecessor is no greater in the case of the tuberculous animal than in the normal. From this point of view the two kinds of stimuli seem to act quite independently and to be capable of summation.

As an incident to our observations as previously reported, we determined the curve of antibody production in the guinea pig for antisheep hemolytic amboceptor. We found it characterized by a double maximum, the first and usual peak at about the 9th day being succeeded by a low point at about the 12th day and a later peak, higher than the first, about the 22nd day. In the course of the present work we have amplified these observations to a certain extent. As shown in Chart 2, the curve as described is followed in its characteristic features as a response to a single injection of *Bacillus typhosus* (killed culture). In this case the second maximum appears somewhat earlier, at the 18th rather than the 22nd day.

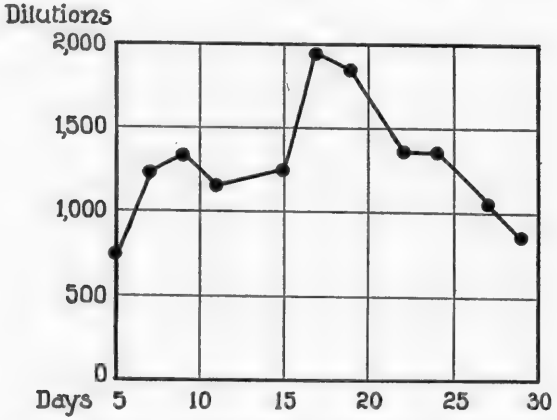

CHART 3. Composite curve of hemolytic amboceptor production in guinea pigs after the fourth of a series of treatments with sheep cells at 28 day intervals.

In such work as we have done with rabbits we have kept this question constantly in mind but have obtained no suggestion of a second peak in them.

In Chart 3 we present the results of observations on a group of guinea pigs for the period succeeding the fourth of a series of injections of sheep red cells at 28 day intervals. It will be noticed that the form of the curve is essentially that of Chart 2 in this paper and those previously published. The first peak of production occurs unchanged at the 9th day. The second peak is advanced to the 17th to 19th day.

As was to be expected, observations made after the last of a series of injections at 5 to 7 day intervals do not develop this form. The high point is usually about the 9th day in such cases, probably because this day following the last injection is also near the second peak of some previous one.

DISCUSSION.

In our previous papers we have defined and used the term "allergic irritability" as a "general characteristic of the animal on the basis of which it reacts to stimuli of the antigenic class, whether they be helpful, injurious, or indifferent to bodily health." The observations herein recorded serve to confirm and amplify the evidence that such a distinction between the actual response of the animal and its "capacity" to respond is of interest. The allergic irritability is found to be increased by a variety of means, particularly by several chronic infections. Infection with the tubercle bacillus under conditions favorable to the full development of its influence is the most effective of these so far as our experience goes.

An intensive treatment with trypan blue throughout the course of the reaction period was almost as effective as infection with the tubercle bacillus. This substance was chosen for experiment because of its well known affinity as a vital stain for cells of the reticuloendothelial cell system particularly the macrophages. There is considerable evidence for the assumption that these cells are increased in number by treatment with trypan blue and related substances. It is likewise to be accepted that cells of this system are greatly proliferated in tuberculosis. Correlation of these suggestions with such evidence as is available as to the origin of antibodies in these cells, and this is also considerable, lends weight to the thought that the stimulation of antibody production which we have observed is based on an increased activity of these cells, due to either an increase in their number or a stimulation of one or more phases of their physiological activity.

There seems to us to be no essential conflict between this conception, or the concrete experiments on which it is based, and the work of others who have succeeded in inhibiting antibody formation by "blocking" these cells with trypan blue and other colloidal substances.

It is, in fact, a rather general rule that substances which exert physiological activity are stimulating in certain doses and depressing when their application is carried to an extreme.

In any event our experiments justify a more serious consideration of allergic irritability as a characteristic, subject to experimental influence in connection with the general principles and phenomena of immunity. It is evidently possible while leaving the specific reactions to antigenic substances intact, to increase greatly their intensity by influences that affect a more general,—one is tempted to say "non-specific,"—set of reactions.

CONCLUSIONS.

1. The allergic irritability of the guinea pig (capacity of the animal to react to antigenic substances) is increased by infection with *Bacillus abortus* and a streptococcus, by the dead tubercle bacillus, and by intensive treatment with trypan blue, respectively. The effect of these influences, while definite, is less pronounced than that previously found for infection with the tubercle bacillus. The production of anti-sheep hemolytic amboceptor was used as the test reaction in these cases.

2. The allergic irritability of the guinea pig with reference to antityphoid agglutinin is increased by infection with the tubercle bacillus.

3. The allergic irritability of the rabbit with reference to anti-sheep hemolytic amboceptor is increased by an infection of suitable severity with the tubercle bacillus.

4. In the guinea pig the curve of antibody production is complex. Its peculiarities are developed during the production of antityphoid agglutinins as well as that of anti-sheep hemolytic amboceptor. In the latter case injections of antigen subsequent to the first give rise to a curve of production unchanged in form but somewhat affected in the time relations.

5. The effects of infection with *Bacillus tuberculosis* on allergic irritability with reference to anti-sheep hemolytic amboceptor are operative throughout a course of immunizing treatments. The successive increases due to the cumulative effect of repeated doses of the antigen are developed on a higher level. The end-result is that the animal with increased irritability furnishes more antibody not

only in response to the initial injection of antigen as previously described, but an absolute increase over the amount attainable by a comparable number of treatments in series. That portion of the final result contributed by the increase in allergic irritability appears to be no less, and may even in instances be somewhat more than that due to the earlier doses of the specific antigen.

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NUCLEAR INCLUSIONS IN THE TESTICLES OF MONKEYS INJECTED WITH THE TISSUE OF HUMAN VARICELLA LESIONS.

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PLATES 5 AND 6.

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Some reasons for studying chicken-pox together with a review of the literature concerning the attempted experimental production of the disease in animals have been given in a previous paper (1). Recently several papers have appeared in support of the view that there is a close relation between chicken-pox and herpes zoster (2) on the one hand and between herpes zoster and herpes simplex (3) on the other. From Doerr's (4) comprehensive review of the literature concerning these diseases one concludes that the proof of the identity of the three or of any two of them is inadequate. Furthermore, it is doubtful if more than one, herpes simplex, has been experimentally produced in animals. Therefore, to solve many of the problems arising in connection with these diseases, it is desirable to establish the other two in animals and this I have attempted to do with varicella.

Early in the work attempts were made to infect monkeys with the virus of chicken-pox. Only Indian macaques were employed and no positive results were obtained. Meantime a filterable virus was discovered during attempts to transmit varicella to rabbits (1). At first this virus (Virus III) was thought to be the etiological agent of chicken-pox. Subsequent work (5), however, in my laboratory and also in Swift's (6) disclosed the fact that Virus III is not the cause of varicella but is indigenous to rabbits. Following this disclosure, the use of monkeys in the experimental study of chicken-pox was resumed and it is with some results of the work that the present paper deals.

Methods and Materials.

Monkeys Employed.—It is well known that species of monkeys vary in susceptibility to certain diseases. Therefore, in addition to Indian macaques (*Macacus rhesus*), African vervets (*Cercopithecus lalandi*) and South American ringtail monkeys (genus *Cebus*) and marmosets (*Hapale jacchus*) were employed. 10 monkeys were inoculated, all of which were young; in none, with the exception of the marmoset, had spermatogenesis been established.

Other Animals.—In addition to the monkeys, 2 rabbits, 1 guinea pig, 1 white rat, and 3 chickens were used.

Inoculations.—Blood, vesicle fluid, and emulsified papules and vesicles collected from patients with varicella, usually during the first 48 hours of the disease, were used for inoculation. Pieces of skin from a normal volunteer were used as a control. The blood was collected and injected before clotting occurred. The fluid from vesicles, to which were added scrapings from the floor of the lesions, was collected in sterile capillary pipettes. The papules and vesicles were excised under aseptic conditions and emulsified by grinding in a mortar moistened with Locke's solution. Sand was not used. The emulsified material was taken up in 0.5–1.0 cc. of Locke's solution and injected by means of a tuberculin syringe. More than 15–20 minutes never elapsed between the collection of the material from the patients and its inoculation into animals. All monkeys were inoculated intratesticularly (0.2–0.25 cc.). In addition to this route, some were inoculated intravenously, intracutaneously (0.1 cc.), and in the inguinal lymph nodes (0.2 cc.). The rabbits, guinea pig, and white rat were inoculated intratesticularly; the chickens in the wattles.

Examination of Animals and Tissues.—Daily examinations of the animals were made for 3 weeks following the inoculations. Tissues, removed under ether anesthesia for histological studies, were fixed in Zenker's fluid with 5 per cent acetic acid, sectioned, and stained with methylene blue and eosin. In addition to a study of the general character of the lesions, a very careful search for eosin-staining nuclear inclusions was made in numerous sections of all the tissues. Details concerning the tinctorial reactions of these inclusions are given by Tyzzer (7), Lipschütz (8), Goodpasture (9), and others.

EXPERIMENTAL.

In the experiments to be reported, the majority of the monkeys, 7 of 10, were vervets. The reason for this will become obvious furthur on and the chief interest in these monkeys centers around the results obtained by means of intratesticular inoculation of emulsified vesicles and papules. In connection with this phase of the work, 14 experiments were performed; and in view of the results obtained it seems advisable to give a detailed account of each.

Experiment 1.—Monkey A; vervet. November 12, 1924. 10 cc. of blood collected from varicella patient, Case 1, 36 hours after the appearance of the eruption, was injected intravenously into Monkey A. Fluid from 30 vesicles was also collected at the same time and injected intradermally in left eyelid, in left and right thighs, and in right side of abdominal wall. While under observation the animal showed no manifestations suggestive of chicken-pox. The temperature ranged from 101.7° to 102.9°F. No tissue was removed for histological study.

Although Monkey A showed no visible specific reaction, it is possible that he became infected and that an immunity to chicken-pox was established. 5 months later he was used for intratesticular inoculation (Experiment 11).

Experiment 2.—Monkey B; vervet. Chicken A. March 5, 1925. 2 papules were removed from varicella patient, Case 2, 18 hours after the appearance of the eruption, emulsified, taken up in 1.0 cc. of Locke's solution, and injected as follows: Monkey B, 0.2 cc. in each testicle, 0.2 cc. in left inguinal lymph node, and 0.1 cc. intradermally; Chicken A, 0.1 cc. in right wattle.

Monkey B.—While under observation the animal showed no gross significant reaction. The temperature ranged from 101.6° to 103.5°F., reaching its highest mark 9 days after the inoculation. A skin nodule, removed on the 5th day, showed a central necrosis surrounded by polymorphonuclear and mononuclear cells. No nuclear inclusions were seen. While the right testicle, removed on the 5th day, showed upon gross examination evidences of injury, nothing of a specific nature was observed. After fixation the testicle was divided into three parts and sections were made of each block. Block 1 showed definite evidences of injury to the tubules and interstitial tissue with an infiltration of mononuclear and polymorphonuclear cells. No nuclear inclusions were found. In Block 2 the reaction was of the same general character as that observed in Block 1, with the exception that it was fairly well localized to a narrow streak running through the section. At one point (Fig. 1) in the damaged interstitial tissue numerous cells, which looked like endothelial leucocytes, were swollen and had within their nuclei typical eosin-staining inclusions (Fig. 6). The inclusions were found in all sections from this block. Block 3: Very little evidence of injury and no nuclear inclusions were observed. The right inguinal lymph node, removed on the 10th day, showed necrotic areas without nuclear inclusions. In the visceral tunic of the left testicle, removed on the 10th day, were found about 20 minute discrete reddish nodules. After fixation the testicle was divided into three parts and sections were made from each block. Blocks 1, 2, and 3: The visceral tunic was thickened and more cellular than usual. In addition to the general thickening, definite localized lesions, made up for the most part of mononuclear cells, were found. The tubules showed very little damage but a few localized lesions were seen in the interstitial

tissue. All the lesions in this testicle were healing and no nuclear inclusions were seen.

Chicken A.—The nodule in the wattle was removed on the 12th day. Upon microscopic examination a central necrosis surrounded by mononuclear cells was found. No nuclear inclusions were observed.

In Experiment 2, typical pink-staining nuclear inclusions were found in the vervet's testicle removed 5 days after inoculation with emulsified varicella papules. None were found, however, in the testicle removed after 10 days.

Experiment 3.—Monkey C; vervet. March 12, 1925. Fluid and scrapings from 20 vesicles were collected from varicella patient, Case 3, 36 hours after the appearance of the rash, mixed with 1.0 cc. of Locke's solution, and injected into Monkey C as follows: 0.25 cc. in each testicle, 0.25 cc. in right inguinal lymph node, and 0.1 cc. intradermally. While under observation the animal showed no significant reaction. The temperature ranged from 101.9° to 103.5°F., reaching its highest point on the 3rd and 13th days after the inoculation. The left testicle was removed on the 11th day, the right on the 13th, and upon gross examination neither showed anything of a specific nature. Each testicle was divided into three parts after fixation and sections were made from each block. The visceral tunic of both was thickened and here and there discrete lesions, characterized by an infiltration of mononuclear cells, were seen. Just beneath the visceral tunic, collections of mononuclear cells were also found. Many of the tubules were damaged and in certain areas of the interstitial tissue there were collections of mononuclear cells. No nuclear inclusions were observed.

The left and right testicle of Monkey C were removed on the 11th and 13th day respectively after inoculation and showed healing lesions in which no nuclear inclusions were seen.

Experiment 4.—Monkey D; vervet. March 23, 1925. 2 skin lesions were removed from varicella patient, Case 4, 48 hours after the appearance of the rash, emulsified, taken up in 0.75 cc. of Locke's solution, and injected into Monkey D as follows: 0.2 cc. in each testicle, 0.1 cc. in right inguinal lymph node, and 0.1 cc. in the skin on the left side of the abdomen. No significant reactions were observed. The temperature ranged from 101.5° to 103.5°F., reaching the highest point on the 4th day after inoculation. The skin nodule and right inguinal lymph node, removed on the 5th day, showed no significant reaction and no nuclear inclusions. The right testicle, removed on the 5th day, upon gross examination showed nothing characteristic. After fixation it was divided into three parts and sections were made from each block. Block 1: The tunic was only slightly damaged. The testicular tissue showed marked evidences of injury with an infiltration of

mononuclear and polymorphonuclear cells in the interstitial tissue. No nuclear inclusions were found. Block 2: The picture was of the same general character as that seen in Block 1. In one area, however, slightly removed from the major portion of the reaction and located just beneath the tunic there was found a discrete lesion (Fig. 2) characterized by hemorrhage and an infiltration of mononuclear cells. Although a few tubules were injured, the principal part of the lesion was in the interstitial tissue and numerous endothelial leucocytes were swollen and contained typical eosin-staining nuclear inclusions (Fig. 3). Block 3: Very little reaction was seen except at one point where there was a definite collection of mononuclear cells. Careful examinations of the left testicle, removed on the 7th day, showed that the inoculum had not entered the gland. No nuclear inclusions were found.

Although no nuclear inclusions were found in the skin and the lymph node removed from Monkey D on the 5th day after inoculation, typical ones were found in the right testicle removed on the same day.

Experiment 5.—Monkey E; vervet. March 24, 1925. 5 lesions, vesicles and papules, were removed from varicella patients—2 from Case 5, 2 from Case 6, and 1 from Case 7—within 48 hours after the appearance of the rash, and ground up together. The emulsified material was taken up in 0.75 cc. of Locke's solution and injected into Monkey E as follows: 0.25 cc. in each testicle, and 0.1 cc. in each of two places in the skin of the abdominal wall. No significant reactions appeared in the animal while under observation. The temperature ranged from 101.6° to 103°F., reaching the highest point on the 1st and 6th days after inoculation. The skin nodule, removed on the 6th day, showed a thickening of the epidermis, and a localized necrosis in the corium surrounded by polymorphonuclear and mononuclear cells. None of the cells contained nuclear inclusions. The left testicle, which was removed on the 6th day, looked as though it had been considerably injured by the inoculation. After fixation it was divided into three parts and sections were made from each block. Blocks 1 and 2: Necrosis of some of the tubules and collections of polymorphonuclear and mononuclear cells in the interstitial tissue were found. Eosin-staining nuclear inclusions were seen in cells of the tubules and in endothelial leucocytes of the interstitial tissue. Block 3: Lesions of the same general character as those seen in Blocks 1 and 2 were encountered. Tubules having cells which contained nuclear inclusions were much more numerous, however. Many of the tubules with swollen cells and nuclear inclusions resembled chicken-pox vesicles (Figs. 4 and 7). Numerous nuclear inclusions were also found in endothelial leucocytes in the interstitial tissue. The right testicle, removed on the 8th day, was also considerably damaged by the inoculation. After fixation it was divided into three parts and sections were made from each block. Extensive damage to the visceral tunic and testicular tissue

with necrosis and infiltration of polymorphonuclear and mononuclear cells was seen in all. No nuclear inclusions were found, however.

Monkey E received the largest inoculum of any animal in the series, getting in all the emulsified tissue of 5 varicella lesions in concentrated form. In this animal for the first and only time I observed nuclear inclusions in glandular cells of the testicle. These eosin-staining bodies were typical of the nuclear inclusions seen in several virus diseases. It is of importance to note that the testicle removed on the 6th day contained many nuclear inclusions while the one removed on the 8th day, although just as badly damaged as the other, showed none. From this it would seem that 8 days is too long a time to wait before castration if one is looking for inclusions.

Experiment 6.—Monkey F; vervet. March 26, 1925. From each of two varicella patients, Cases 8 and 9, a papule and a vesicle were removed within 48 hours after the appearance of the eruption, emulsified, taken up in 1.0 cc. of Locke's solution, and injected into Monkey F as follows: 0.25 cc. in each testicle, and 0.1 cc. in each of three places in the skin over the abdomen. No significant reaction appeared in the animal while under observation. The temperature ranged between 102° and 103.8°F., reaching the highest point 24 hours after inoculation. A skin nodule was removed on the 5th and the 7th days. Upon gross and microscopic examination these showed nothing of a specific nature. The left testicle, removed on the 5th day, was nodular, and hemorrhagic spots were seen in the tunic. After fixation the testicle was divided into three parts and sections were made from each block. Block 1: Considerable damage to the tunic and the testicular tissue had resulted from the inoculation. There was nothing specific in the reaction as a whole. In one area, however, numerous endothelial leucocytes were swollen and contained eosin-staining nuclear inclusions. Block 2: The reaction was in general similar to that in Block 1 but there were no nuclear inclusions. Block 3: The reaction was of the same general character as that seen in Blocks 1 and 2. In one area in the interstitial tissue somewhat removed from the point of maximal damage, there was a collection of endothelial leucocytes some of which contained typical pink nuclear inclusions. The right testicle, removed on the 7th day, did not seem to be damaged. Sections from three blocks showed that the inoculum had not entered the gland. The testicular tissue was normal and no inclusions were found. Sections of the epididymis showed considerable damage with collections of polymorphonuclear and mononuclear cells. No inclusions were found, however.

Nuclear inclusions were found in two of three blocks from the left testicle of Vervet F removed 5 days after inoculation. No inclu-

sions were found in pieces of skin removed 5 and 7 days following the inoculation.

Experiment 7.—Chickens B and C. March 27, 1925. 2 recently formed vesicles were removed from varicella patient, Case 10, within the first 48 hours of the disease, emulsified, and taken up in 1.0 cc. of Locke's solution. 0.2 cc. of the emulsion was injected into each wattle of two chickens, B and C. A wattle was removed on the 4th, 5th, and 7th days after inoculation. Nothing specific was observed upon gross or upon microscopic examination. No nuclear inclusions were seen.

Experiment 8.—Monkey G; ringtail. April 2, 1925. From varicella patient, Case 11, 3 lesions, 1 papule and 2 vesicles, were removed within 36 hours after the onset of the disease, emulsified, taken up in 0.75 cc. of Locke's solution, and injected into Monkey G as follows: 0.2 cc. in each testicle and 0.1 cc. in each of 2 areas in the skin of the abdominal wall. While under observation the animal showed nothing of a specific nature. The temperature ranged from 102° to 103.5°F., reaching the highest point 24 hours after inoculation. A skin nodule and both testicles were removed on the 5th day. Each testicle was divided into three parts and sections were made from each block. In sections of the skin the epidermis was thickened and the corium was necrotic in places and contained many mononuclear and polymorphonuclear cells. No inclusions were seen. Both testicles had been injured by the inoculation and showed necrosis, hemorrhage, and collections of mononuclear and polymorphonuclear cells. No nuclear inclusions were found.

Experiment 9.—Guinea Pig A. April 3, 1925. A papule and a vesicle were removed from varicella patient, Case 11, on the 3rd day of the disease, emulsified, taken up in 0.5 cc. of Locke's solution, and injected into the testicles of Guinea Pig A. Nothing of a specific nature was observed in the animal while under observation. The temperature ranged from 100.8° to 102.2°F. Both testicles, removed on the 5th day, were divided into three parts each after fixation, and sections were made from each block. Both testicles had been damaged by the inoculation. There were areas of necrosis surrounded by hemorrhage and collections of mononuclear and polymorphonuclear cells. No nuclear inclusions were seen.

Experiment 10.—Rabbit A. April 5, 1925. A papule and a vesicle were removed from varicella patient, Case 12, 36 hours after the appearance of the rash, emulsified, taken up in 0.5 cc. of Locke's solution, and injected into the left testicle of Rabbit A. Nothing specific was noted in the animal while under observation. Its temperature ranged from 102° to 103.5°F., reaching the highest point 4 days after the inoculation. Both testicles were removed on the 5th day. After fixation the right was divided into four parts and the left into five. Sections were made from each block. The right uninoculated testicle proved normal. The left testicle had been considerably damaged by the inoculation but no nuclear inclusions were found.

Experiment 11.—Monkey A, vervet; Monkey H, macaque; White Rat A. April 7, 1925. 24 hours after the appearance of the eruption, 5 lesions were removed from 2 varicella patients—1 vesicle and 2 papules from Case 13 and 2 papules from Case 14. The material was emulsified, taken up in 1.0 cc. of Locke's solution, and injected, into 3 animals as follows: 0.25 cc. in the right testicle of Monkey A; 0.15 cc. in each testicle and 0.1 cc. in the skin of the abdominal wall of Monkey H; and 0.1 cc. in each testicle of White Rat A.

Monkey A, Vervet.—The right testicle, removed on the 6th day, was divided into three parts after fixation. Sections made from each block showed that considerable damage to the tubules and interstitial tissue had resulted from the inoculation. No nuclear inclusions were found.

Monkey H, Macaque.—The left and right testicle, removed on the 4th and 6th day respectively, were divided and sectioned in the usual manner. Considerable damage to the tubules and interstitial tissue had resulted from the inoculation but no nuclear inclusions were found.

White Rat A.—Both testicles, removed on the 6th day, were divided and sectioned in the usual manner. Although considerable damage to the tubules and interstitial tissue had resulted from the inoculation, no nuclear inclusions were seen.

Monkey A, vervet, had already been used in Experiment 1 and at the time of inoculation might have been immune to varicella because of the previous injection. At any rate no nuclear inclusions were found in the right testicle 6 days after inoculation.

Experiment 12.—Monkey I; marmoset. April 8, 1925. 2 vesicles and 1 papule were removed from varicella patient, Case 15, within 48 hours after the appearance of the rash, emulsified, taken up in 0.5 cc. of Locke's solution, and injected into Monkey I, 0.15 cc. in each testicle. Both testicles were removed on the 5th day and after fixation each one was divided into three parts. Sections from each block showed the damage to the tubules and interstitial tissue which is an inevitable result of the inoculation, but no nuclear inclusions were found.

Experiment 13.—Rabbit B. April 9, 1925. 1 vesicle and 1 papule were removed from varicella patient, Case 16, about 36 hours after the appearance of the eruption. The material was emulsified, taken up in 0.75 cc. of Locke's solution, and injected into the right testicle of Rabbit B. This testicle was removed on the 4th day and after fixation was divided into seven parts. Sections were made from each block. Considerable damage to the tubules and interstitial tissue had resulted from the inoculation, but no nuclear inclusions were found.

Experiment 14.—Monkey J; vervet. April 11, 1925. 2 pieces of skin were removed from a normal adult volunteer, emulsified, taken up in 0.4 cc. of Locke's solution, and injected into Monkey J, 0.2 cc. in each testicle. The temperature of the animal ranged from 101.8° to 104°F., reaching the highest point 48 hours

TABLE I.

Occurrence of the Nuclear Inclusions.

Experimental animal.	Inoculum.	Tissues examined histologically.	No. of days after inoculation.	Nuclear inclusions.
Vervet A (immune ?).	Emulsified lesions.	Right testicle.	6	−
Vervet B	" "	Skin.	5	−
		Right testicle.	5	+
		Left "	10	−
		Inguinal lymph node.	10	−
" C	Vesicle fluid and scrapings.	Left testicle.	11	−
		Right "	13	−
" D	Emulsified lesions.	Skin.	5	−
		Inguinal lymph node.	5	−
		Right testicle.	5	+
" E	" "	Skin.	6	−
		Left testicle.	6	+
		Right "	8	−
" F	" "	Skin.	5	−
		"	7	−
		Left testicle.	5	+
" J	Skin from normal volunteer.	Right "	5	−
Ringtail G	Emulsified lesions.	Skin.	5	−
		Right testicle.	5	−
		Left "	5	−
Macaque H	" "	Right "	4	−
		Left "	6	−
Marmoset I	" "	Right "	5	−
		Left "	5	−
Rabbit A	" "	Left "	5	−
" B	" "	Right "	4	−
Guinea Pig A	" "	" "	5	−
		Left "	5	−

TABLE I—*Concluded.*

Experimental animal.	Inoculum.	Tissues examined histologically.	No. of days after inoculation.	Nuclear inclusions.
White Rat A	Emulsified lesions.	Right testicle.	6	—
		Left "	6	—
Chicken A	" "	Right wattle.	12	—
" B	" "	" "	4	—
		Left "	5	—
" C	" "	Right "	7	—

+ indicates presence of eosin-staining nuclear inclusions.
— " absence " " " " "
Testicles into which the inoculum did not enter are not indicated in this table.

after inoculation. The right testicle, removed on the 5th day, was divided into three parts after fixation. Sections from each block showed that damage to the tubules and interstitial tissue had resulted from the inoculation. This damage was characterized by necrosis, hemorrhage, and collections of mononuclear and polymorphonuclear cells. No nuclear inclusions were found. The left testicle, removed on the 7th day, was normal since the inoculum had not entered the gland. No inclusions were found.

A summary of the experiments with special reference to the presence of eosin-staining nuclear inclusions in the inoculated tissues is given in Table I.

DISCUSSION.

A number of diseases are characterized by fever, papules and vesicles showing some destruction of the skin and infiltration of it with polymorphonuclear and mononuclear cells. In some of them, *e.g.* chicken-pox, herpes simplex, and herpes zoster, the gross and microscopic appearance of the skin lesions are so similar as to be almost identical. In addition to this, eosin-staining nuclear inclusions (Fig. 5) in the epidermal cells and endothelial leucocytes are a most characteristic feature. Furthermore, tissues experimentally damaged by the virus of herpes simplex regularly show acidophilic nuclear bodies (10). Similar inclusions are also found in lesions produced by Virus III (11) which is indigenous to rabbits.

The eosin-staining nuclear inclusions are so consistently found under certain conditions that to many workers they appear extremely significant. Some consider them merely as products of degeneration, but others believe that they are the virus itself, while yet others think of them as virus surrounded by a mantle of altered nuclear material. As yet their nature has not been definitely determined. This fact, however, does not lessen their significance for experimental work. I have never found typical, eosin-staining nuclear inclusions save in virus diseases, and this has been the experience of other workers (12). In view of this fact there is reason to believe that infection with a virus has taken place when such inclusions are found (10). To find them is not always easy, especially in experimental animals, and a careful search should be made before a negative result is recorded. Furthermore, it must be kept in mind that there is an optimum time (9) following the inoculation for the finding of the inclusions after which they disappear rapidly.

For the 14 experiments reported in this paper, material from 16 typical cases of chicken-pox was used. The wide derivation of the material lessens the chances of an incorrect diagnosis affecting the results. The nuclear inclusions were found only in the testicles of 4 vervets,—which had been inoculated with material from different sources,—and not in other inoculated tissues of the same animals, nor anywhere in the other experimental animals (Table I). Furthermore, they were not found in the testicles of a vervet inoculated with normal skin, in the testicle of a vervet inoculated with emulsified varicella lesions but possibly immunized by a previous injection, nor in a vervet's testicles removed 11 and 13 days after inoculation with vesicle fluid and scrapings. The testicles of the 4 vervets in which inclusions were found were removed 5 and 6 days after inoculation while those removed after 8 and 10 days, although inoculated with the same material and as severely traumatized as the others, contained no inclusions. It is of importance that the 4 vervets in which positive results were obtained came from at least two sources and were not in contact with one another at any time. In the light of what is known concerning eosin-staining nuclear inclusions and in view of the conditions under which the experiments reported in this paper were performed, it seems reasonable to conclude that the acido-

philic nuclear inclusions in the 4 vervets' testicles were manifestations of the presence of a virus. The nature of the virus and the possibility of transmitting it through a series of monkeys remain to be determined.

CONCLUSIONS.

Eosin-staining nuclear inclusions resembling those deemed characteristic of a certain well known group of filterable viruses, amongst which is varicella, were found in vervets' testicles inoculated with emulsified tissue of human varicella lesions.

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EXPLANATION OF PLATES.

PLATE 5.

FIG. 1. Lesion in right testicle of Vervet B following the injection of emulsified varicella material. *A* indicates area from which Fig. 6 was drawn. × 135.

FIG. 2. Discrete lesion in the right testicle of Vervet D 5 days after inoculation with varicella material. *A* indicates area from which photograph in Fig. 3 was taken. × 135.

FIG. 3. Nuclear inclusions indicated by *B* in the lesion shown in Fig. 2. × 1000.

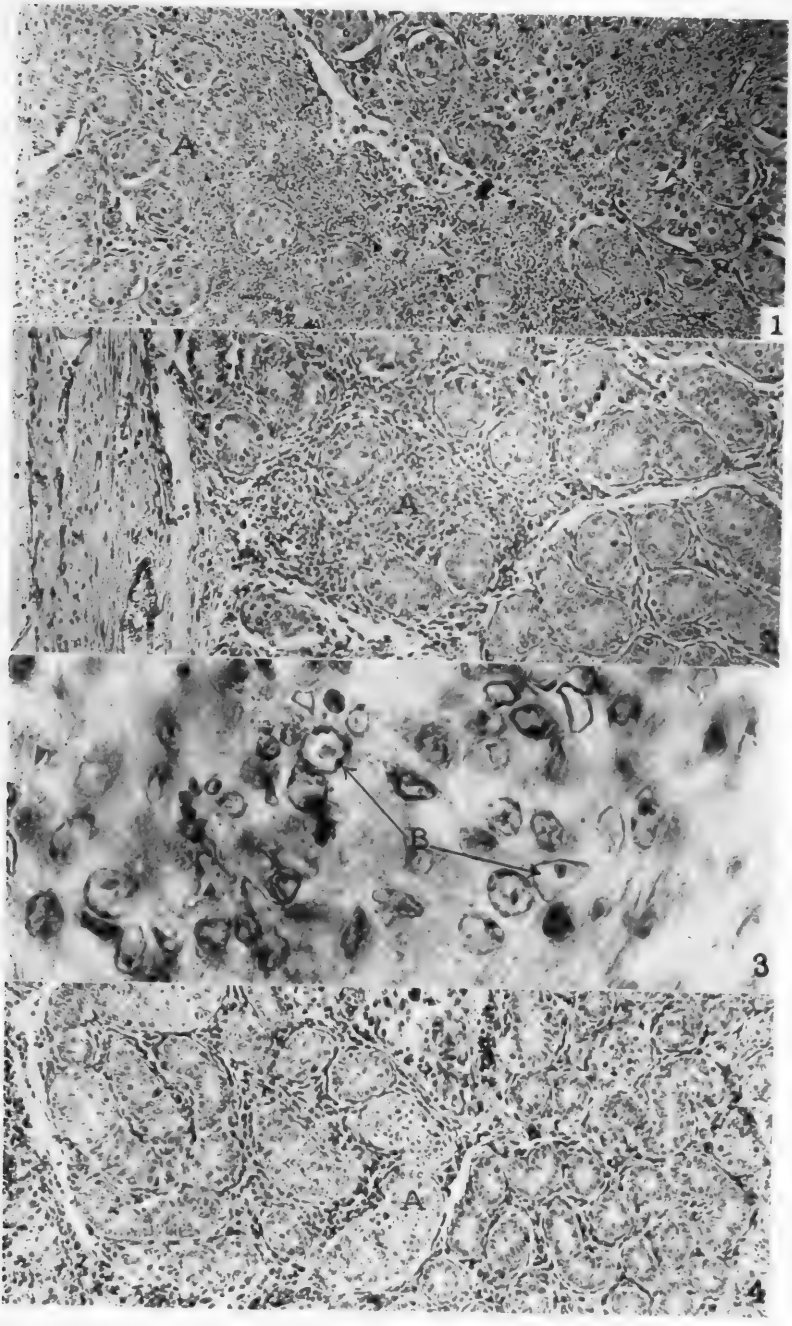
FIG. 4. Lesion in left testicle of Vervet E following the injection of emulsified varicella material. *A* indicates area from which Fig. 7 was drawn. × 135.

PLATE 6.

FIG. 5. Nuclear inclusions in epidermal cells of human varicella lesion. Eosin-methylene blue. × 600.

FIG. 6. Nuclear inclusions in endothelial leucocytes of right testicle of Vervet B. Eosin-methylene blue. × 600. × 1200.

FIG. 7. Nuclear inclusions in gland cells of the left testicle of Vervet E. Eosin-methylene blue. × 600. × 1200.



(Rivers: Varicella.)

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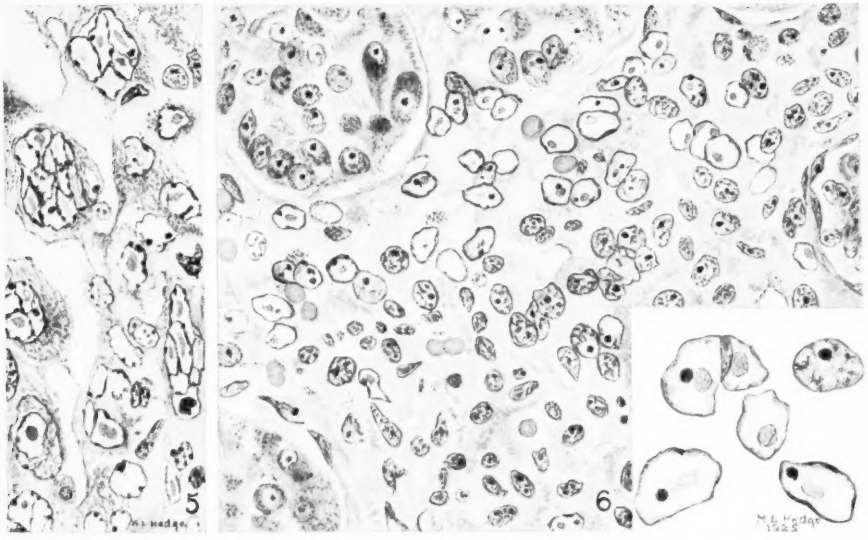

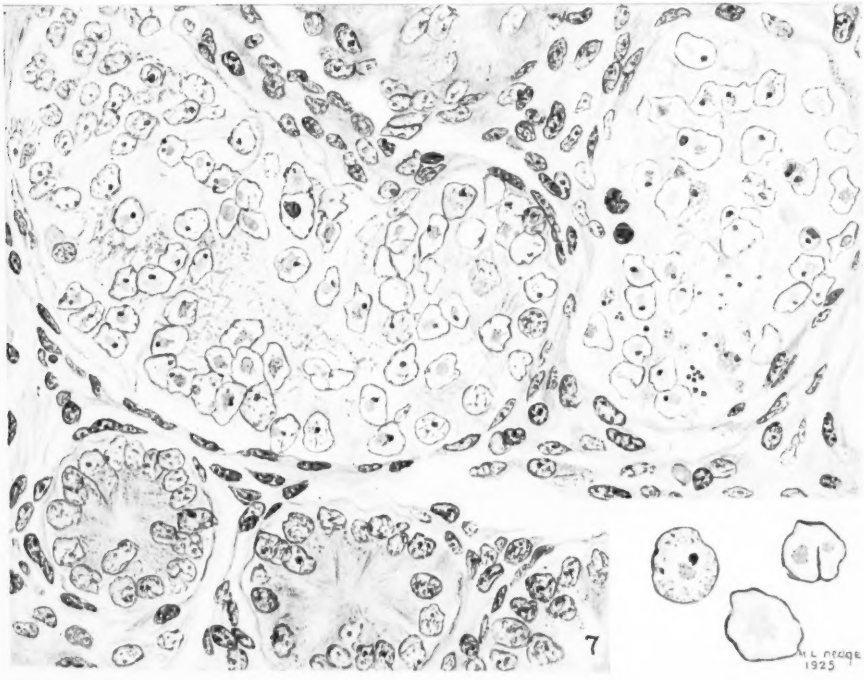

(Rivers: Varicella.)

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